generate the mature enzyme. Based on these lines of evidence, it is proposed that cathepsin L is recognized by mechanisms similar to those for the intracellular sorting and processing of vacuolar proteins in the yeast cells.

```
=> d his

     (FILE 'HOME' ENTERED AT 08:17:45 ON 03 APR 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 08:18:06 ON
     03 APR 2002
L1          3858 S SINGLE CHAIN ANTIBODY
L2            33 S L1 AND "PHOX"
L3            10 DUP REMOVE L2 (23 DUPLICATES REMOVED)
L4             0 S L2 AND MEMBRANE PERMEANT PROBE
L5             0 S "PHOX BODIYP FL"
L6             0 S "PHOX BODIPY FL"
L7        718845 S PROBE
L8          6415 S L7 AND CONJUGATE
L9             6 S L8 AND PHOX
L10            2 DUP REMOVE L9 (4 DUPLICATES REMOVED)
L11          146 S FARINAS J?/AU
L12            0 S L11 AND INTRACELLULAR FLUORESCENT PROBE
L13            6 S L11 AND SINGLE CHAIN ANTIBODY
L14            2 DUP REMOVE L13 (4 DUPLICATES REMOVED)
L15         1400 S INTRACELLULAR TARGETING
L16           24 S L15 AND SINGLE CHAIN
L17            0 S L16 AND PROBE
L18           11 DUP REMOVE L16 (13 DUPLICATES REMOVED)

=> dup remove l11
PROCESSING COMPLETED FOR L11
L19           70 DUP REMOVE L11 (76 DUPLICATES REMOVED)

=> s l19 and "PhOx conjugate"
L20            0 L19 AND "PHOX CONJUGATE"

=> s l19 and ligand conjugate
L21            1 L19 AND LIGAND CONJUGATE

=> d l21 cbib abs

L21  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2002 ACS
1999:659579   Document No. 131:283619  Methods and reagents for targeting
     organic compounds to selected cellular locations.  Farinas, Javier
     (The Regents of the University of California, USA).  PCT Int. Appl. WO
     9951986 A1 19991014, 69 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU,
     AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD,
     GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
     LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG,
     SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY,
     KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE,
     DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN,
     TD, TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1999-US7847 19990408.
     PRIORITY: US 1998-81118 19980408; US 1998-81340 19980409.
AB   The present invention provides methods and reagents for targeting probes
     to selected cellular locations, through the expression of specific binding
     partners to that probe within the cell.  In one embodiment, the probes may
     comprise spectroscopic probes that can be used in a method for localizing
     a specific binding partner within a cell, and for creating assays for
     post-translational activities.  The invention allows the monitoring of the
     location of such intracellular specific binding partners over time and in
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response to stimuli, such as test chems. The spectroscopic probes can be used for screening a test chem. for activity. The present invention also includes cells and transgenic organisms comprising the intracellular specific binding partner, wherein the specific binding partner can bind with the spectroscopic probe/**ligand conjugate**. CHO cells were transfected with cDNAs encoding single chain antibody (sFv) fusion products with a Golgi-targeting human .beta.-1,4-galactosyltransferase fragment. The sFv bound to hapten 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (phOx)-fluorescein conjugate. The Golgi-targeted phOx-fluorescein was used to detect continuous changes in lumenal pH in individual cells.

```
=> s spectroscopic probe
L22       1533 SPECTROSCOPIC PROBE

=> s l22 and cell permeant
   3 FILES SEARCHED...
L23          0 L22 AND CELL PERMEANT

=> s l22 and Bodipy Fl
L24          0 L22 AND BODIPY FL

=> s cell expressing single chain antibody
   2 FILES SEARCHED...
   4 FILES SEARCHED...
L25          1 CELL EXPRESSING SINGLE CHAIN ANTIBODY

=> d l25 cbib abs

L25  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2002 ACS
2000:450926   Document No. 134:84694  Prostate-specific membrane antigen
     (PSMA)-specific monoclonal antibodies in the treatment of prostate and
     other cancers.  Gong, Michael C.; Chang, Sam S.; Sadelain, Michel; Bander,
     Neil H.; Heston, Warren D. W. (Department of Urology, Memorial
     Sloan-Kettering Cancer Center, New York, NY, USA).  Cancer and Metastasis
     Reviews, Volume Date 1999, 18(4), 483-490 (English) 2000.  CODEN: CMRED4.
     ISSN: 0167-7659.  Publisher: Kluwer Academic Publishers.
AB   A review with 35 refs.  Prostate-specific membrane antigen (PSMA) is a
     cell surface glycoprotein that is expressed by prostate epithelial cells.
     PSMA-specific monoclonal antibodies have been utilized to characterize the
     biol. function and in vivo biodistribution of PSMA.  PSMA is an attractive
     target protein for monoclonal antibody directed imaging or therapeutics
     for prostate cancer since its expression is relatively restricted to
     prostate epithelial cells and is over-expressed in prostate cancer,
     including in advanced stages.  Currently, clin. usage of PSMA specific
     monoclonal antibodies has been limited to diagnostic immunohistochem. and
     imaging of patients with prostate cancer.  Novel applications for these
     antibodies will be discussed.

=> s method
L26    9878122 METHOD

=> s l26 and localizing probe
L27          6 L26 AND LOCALIZING PROBE

=> dup remove l27
PROCESSING COMPLETED FOR L27
L28           4 DUP REMOVE L27 (2 DUPLICATES REMOVED)

=> d l28 1-4 cbib abs
```

L28 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2002 ACS
1997:369754 Document No. 126:339662 Single molecule detection by in situ hybridization and a digital imaging fluorescence microscopy system. Singer, Robert H.; Femino, Andrea M.; Fogarty, Kevin E. (University of Massachusetts, USA). PCT Int. Appl. WO 9714816 A1 19970424, 49 pp. DESIGNATED STATES: W: AU, CA, JP, KR, MX; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US16811 19961021. PRIORITY: US 1995-546072 19951020.

AB Disclosed are **methods** for accurately detg. the total fluorescence intensity (TFI) of a single fluorochrome, under imaging conditions, using a digital imaging fluorescence microscopy system. Also are **methods** for detecting and **localizing probe** -target mol. binding. The detection **methods** have sufficient resoln. and sensitivity to locate and detect a single target-bound probe bound to a target mol. that can be as short as 20 nucleotides. The **method** is useful in diagnosis for the infection by, e.g., HIV. The **method** was demonstrated by detection of .beta.- and .gamma.-actin mRNA by using .beta.- and .gamma.-actin 3'-UTR probes labeled with fluorescein and CY3, resp., and obsd. with a Nikon DIAPHOT inverted epifluorescence microscope that is capable of digital imaging.

L28 ANSWER 2 OF 4 MEDLINE DUPLICATE 1
96265948 Document Number: 96265948. PubMed ID: 8661803. Endoscopic surgery of the rhinobasis with a computer-assisted localizer. Kruckels G; Korves B; Klimek L; Mosges R. (Department of Ear, Nose and Throat Surgery, Plastic Head and Neck Surgery, Medical Faculty, Technical University of Aachen, Germany.) SURGICAL ENDOSCOPY, (1996 Apr) 10 (4) 453-6. Journal code: VBF; 8806653. ISSN: 0930-2794. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The endoscope is useful for the diagnosis and surgical therapy of diseases of the nose, the paranasal sinuses and its neighboring regions, and allows for microinvasive, functional approaches. The reduced invasiveness of therapeutic procedures is sometimes accompanied by insufficient clearness of the surgical field, however. This significant problem is solved by the computer-assisted-surgery (CAS) system, an intraoperative localizer. It allows continuous orientation based on three-dimensional reconstructed preoperative CT scans with superimposed positioning of the endoscope. We have now adapted CAS for endoscopic sinus surgery, which meant that a variety of visualization **methods** were tested. A conventional straightforward endoscope was used in combination with, or as, the **localizing probe**. A dual-display technique was adjusted to video-endoscopic procedures: the information of the localizer is displayed on one monitor while the video-endoscopic picture is viewed on a second screen. In addition, a single-display technique with both images on one monitor was developed. It proved to be the most promising way of combining endoscopy and intraoperative CT-image-guided localization.

L28 ANSWER 3 OF 4 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
94308665 EMBASE Document No.: 1994308665. [Functional endoscopic surgery of the rhinobasis with computer assisted localizer]. ENDOSKOPISCHE NASENNEBENHOHLENCHIRURGIE MIT COMPUTERUNTERSTUTZTER LOKALISATIONSHILFE. Korves B.; Kruckels G.; Klimek L.; Mosges R.. Klinik fur HNO-Heilkunde, Plastische Kopf- und Halschirurgie, Medizinische Fakultat, Pauwelsstrasse 30,D-52057 Aachen, Germany. Oto-Rhino-Laryngologia Nova 4/3 (164-167) 1994.
ISSN: 1014-8221. CODEN: OTNOEQ. Pub. Country: Switzerland. Language: German. Summary Language: German; English.

AB Use of the endoscope can be beneficial for the diagnosis and therapy of diseases of the nose, the paranasal sinuses and its neighboring anatomical regions. However, there is a trade-off between the reduced invasiveness of therapeutic procedures and the reduced clearness of the operative site. We have adapted the Aachen Computer-Assisted Surgery system for use in endoscopic sinus surgery. A variety of visualization **methods** has

been tested. With conventional direct view endoscopy, the endoscope can be used in combination with a **localizing probe** or as the **localizing probe** itself. In video-endoscopic procedures a duplex-display technique may be used, where the information of the localizer is displayed on one monitor while the video-endoscopic picture is viewed on a second screen. With the single-display technique, the information derived from online intraoperative localization and from the video-endoscopy are integrated into one monitor screen. The device has been used in 23 cases of endoscopic sinus procedures.

L28 ANSWER 4 OF 4 MEDLINE DUPLICATE 2

80223465 Document Number: 80223465. PubMed ID: 6156040. Fluorescent probes to detect lymphocyte activation. Nairn R C; Rolland J M. CLINICAL AND EXPERIMENTAL IMMUNOLOGY, (1980 Jan) 39 (1) 1-13. Ref: 157. Journal code: DD7; 0057202. ISSN: 0009-9104. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Fluorescent probes can monitor events in lymphocytes stimulated by mitogens and antigens. Early activation is associated with conformational changes in membrane macromolecules, and has been studied by measurement of fluorescence intensity or polarization of the membrane-**localizing probes** ANS, NPN, DPH and TMRITC. Subsequent changes in cytoplasmic macromolecules have been detected by altered fluorescence polarization of intracellular fluorescein. Altered metabolic activity in the activated lymphocyte is also revealed by fluorescent probes: the increased red fluorescence of lysosomes seen by AO staining, is attributable to altered lysosome membrane permeability. AO fluorescence has also detected early changes in the nuclear nucleoprotein complex. The later synthesis of new DNA is readily demonstrated by increased staining with the nuclear probes AO, ethidium bromide, propidium iodide, mithramycin and the Hoechst dyes. Adaptation of fluorescent probe analyses to the now rapidly developing flow microfluorimeters is providing rapid and sensitive assays of lymphocyte stimulation. Such **methods** will permit routine detection of lymphocyte response to particular antigens or mitogens, as well as identification of antigenic substances by their stimulation of known reactive lymphocytes. Last but not least, fluorescent probes are providing new understanding of the cellular events and regulatory mechanisms associated with lymphocyte activation.

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NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Apr 08	"Ask CAS" for self-help around the clock
NEWS 3	Apr 09	BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS 4	Apr 09	ZDB will be removed from STN
NEWS 5	Apr 19	US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 6	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 7	Apr 22	BIOSIS Gene Names now available in TOXCENTER
NEWS 8	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS 9	Jun 03	New e-mail delivery for search results now available
NEWS 10	Jun 10	MEDLINE Reload
NEWS 11	Jun 10	PCTFULL has been reloaded
NEWS 12	Jul 02	FOREGE no longer contains STANDARDS file segment
NEWS 13	Jul 22	USAN to be reloaded July 28, 2002; saved answer sets no longer valid
NEWS 14	Jul 29	Enhanced polymer searching in REGISTRY
NEWS 15	Jul 30	NETFIRST to be removed from STN
NEWS 16	Aug 08	CANCERLIT reload
NEWS 17	Aug 08	PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 18	Aug 08	NTIS has been reloaded and enhanced
NEWS 19	Aug 19	Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 20	Aug 19	IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS 21	Aug 19	The MEDLINE file segment of TOXCENTER has been reloaded
NEWS 22	Aug 26	Sequence searching in REGISTRY enhanced
NEWS 23	Sep 03	JAPIO has been reloaded and enhanced
NEWS 24	Sep 16	Experimental properties added to the REGISTRY file
NEWS 25	Sep 16	CA Section Thesaurus available in CAPLUS and CA
NEWS 26	Oct 01	CASREACT Enriched with Reactions from 1907 to 1985
NEWS 27	Oct 21	EVENTLINE has been reloaded
NEWS 28	Oct 24	BEILSTEIN adds new search fields
NEWS 29	Oct 24	Nutraceuticals International (NUTRACEUT) now available on STN
NEWS 30	Oct 25	MEDLINE SDI run of October 8, 2002
NEWS 31	Nov 18	DKILIT has been renamed APOLLIT
NEWS 32	Nov 25	More calculated properties added to REGISTRY
NEWS 33	Dec 02	TIBKAT will be removed from STN
NEWS 34	Dec 04	CSA files on STN
NEWS 35	Dec 17	PCTFULL now covers WP/PCT Applications from 1978 to date
NEWS 36	Dec 17	TOXCENTER enhanced with additional content
NEWS 37	Dec 17	Adis Clinical Trials Insight now available on STN
NEWS 38	Dec 30	ISMEC no longer available
NEWS 39	Jan 21	NUTRACEUT offering one free connect hour in February 2003
NEWS 40	Jan 21	PHARMAML offering one free connect hour in February 2003
NEWS 41	Jan 29	Simultaneous left and right truncation added to COMPENDEX, ENERGY, INSPEC
NEWS 42	Feb 13	CANCERLIT is no longer being updated
NEWS 43	Feb 24	METADEX enhancements
NEWS 44	Feb 24	PCTGEN now available on STN
NEWS 45	Feb 24	TEMA now available on STN

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NEWS 46  Feb 26  NTIS now allows simultaneous left and right truncation
NEWS 47  Feb 26  PCTFULL now contains images
NEWS 48  Mar 04  SDI PACKAGE for monthly delivery of multifile SDI results
NEWS 49  Mar 19  APOLLIT offering free connect time in April 2003
NEWS 50  Mar 20  EVENTLINE will be removed from STN
NEWS 51  Mar 24  PATDPAFULL now available on STN
NEWS 52  Mar 24  Additional information for trade-named substances without
                 structures available in REGISTRY
NEWS 53  Mar 24  Indexing from 1957 to 1966 added to records in CA/CAPLUS

NEWS EXPRESS  April 4 CURRENT WINDOWS VERSION IS V6.01a, CURRENT
              MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
              AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
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NEWS INTER    General Internet Information
NEWS LOGIN    Welcome Banner and News Items
NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
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=> s localizing probe
L1            8 LOCALIZING PROBE

=> dup remove l1
PROCESSING COMPLETED FOR L1
L2            6 DUP REMOVE L1 (2 DUPLICATES REMOVED)

=> d l2 1-6 cbib abs

L2   ANSWER 1 OF 6  CAPLUS  COPYRIGHT 2003 ACS
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1997:369754 Document No. 126:339662 Single molecule detection by in situ hybridization and a digital imaging fluorescence microscopy system. Singer, Robert H.; Femino, Andrea M.; Fogarty, Kevin E. (University of Massachusetts, USA). PCT Int. Appl. WO 9714816 A1 19970424, 49 pp. DESIGNATED STATES: W: AU, CA, JP, KR, MX; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US16811 19961021. PRIORITY: US 1995-546072 19951020.

AB Disclosed are methods for accurately detg. the total fluorescence intensity (TFI) of a single fluorochrome, under imaging conditions, using a digital imaging fluorescence microscopy system. Also are methods for detecting and **localizing probe**-target mol. binding. The detection methods have sufficient resoln. and sensitivity to locate and detect a single target-bound probe bound to a target mol. that can be as short as 20 nucleotides. The method is useful in diagnosis for the infection by, e.g., HIV. The method was demonstrated by detection of .beta.- and .gamma.-actin mRNA by using .beta.- and .gamma.-actin 3'-UTR probes labeled with fluorescein and CY3, resp., and obsd. with a Nikon DIAPHOT inverted epifluorescence microscope that is capable of digital imaging.

L2 ANSWER 2 OF 6 MEDLINE DUPLICATE 1

96265948 Document Number: 96265948. PubMed ID: 8661803. Endoscopic surgery of the rhinobasis with a computer-assisted localizer. Kruckels G; Korves B; Klimek L; Mosges R. (Department of Ear, Nose and Throat Surgery, Plastic Head and Neck Surgery, Medical Faculty, Technical University of Aachen, Germany.) SURGICAL ENDOSCOPY, (1996 Apr) 10 (4) 453-6. Journal code: 8806653. ISSN: 0930-2794. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The endoscope is useful for the diagnosis and surgical therapy of diseases of the nose, the paranasal sinuses and its neighboring regions, and allows for microinvasive, functional approaches. The reduced invasiveness of therapeutic procedures is sometimes accompanied by insufficient clearness of the surgical field, however. This significant problem is solved by the computer-assisted-surgery (CAS) system, an intraoperative localizer. It allows continuous orientation based on three-dimensional reconstructed preoperative CT scans with superimposed positioning of the endoscope. We have now adapted CAS for endoscopic sinus surgery, which meant that a variety of visualization methods were tested. A conventional straightforward endoscope was used in combination with, or as, the **localizing probe**. A dual-display technique was adjusted to video-endoscopic procedures: the information of the localizer is displayed on one monitor while the video-endoscopic picture is viewed on a second screen. In addition, a single-display technique with both images on one monitor was developed. It proved to be the most promising way of combining endoscopy and intraoperative CT-image-guided localization.

L2 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

1995:91394 Document No.: PREV199598105694. Computer-assisted **localizing probe** using 3-D CT/MRI images for skull base surgery. Mattox, Douglas E. (1); Long, Donlin; Bryan, Nick; Legget, Bruce; Zinreich, S. James. (1) Dep. Otolaryngol., Univ. Md., Baltimore, MD 21201 USA. Samii, M. [Editor]. (1994) pp. 40-43. Skull base surgery: Anatomy, diagnosis and treatment. Publisher: S. Karger AG P.O. Box, Allschwilerstrasse 10, CH-4009 Basel, Switzerland. Meeting Info.: First International Skull Base Congress Hannover, Germany June 14-20, 1992 ISBN: 3-8055-5967-4. Language: English.

L2 ANSWER 4 OF 6 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

94308665 EMBASE Document No.: 1994308665. [Functional endoscopic surgery of the rhinobasis with computer assisted localizer]. ENDOSKOPISCHE NASENNEBENHOHLENCHIRURGIE MIT COMPUTERUNTERSTUTZTER LOKALISATIONSHILFE. Korves B.; Kruckels G.; Klimek L.; Mosges R.. Klinik fur HNO-Heilkunde, Plastische Kopf- und Halschirurgie, Medizinische Fakultat, Pauwelsstrasse

30,D-52057 Aachen, Germany. Oto-Rhino-Laryngologia Nova 4/3 (164-167) 1994.
ISSN: 1014-8221. CODEN: OTNOEQ. Pub. Country: Switzerland. Language: German. Summary Language: German; English.

AB Use of the endoscope can be beneficial for the diagnosis and therapy of diseases of the nose, the paranasal sinuses and its neighboring anatomical regions. However, there is a trade-off between the reduced invasiveness of therapeutic procedures and the reduced clearness of the operative site. We have adapted the Aachen Computer-Assisted Surgery system for use in endoscopic sinus surgery. A variety of visualization methods has been tested. With conventional direct view endoscopy, the endoscope can be used in combination with a **localizing probe** or as the **localizing probe** itself. In video-endoscopic procedures a duplex-display technique may be used, where the information of the localizer is displayed on one monitor while the video-endoscopic picture is viewed on a second screen. With the single-display technique, the information derived from online intraoperative localization and from the video-endoscopy are integrated into one monitor screen. The device has been used in 23 cases of endoscopic sinus procedures.

L2 ANSWER 5 OF 6 MEDLINE

91089428 Document Number: 91089428. PubMed ID: 2264220. [Significance of topographic diagnosis of foreign bodies situated in the orbit]. Znachenie topograficheskoi diagnostiki inorodnykh tel, raspolozhennykh v orbite. Gundarova R A; Kataev M G. VESTNIK OFTALMOLOGII, (1990 Sep-Oct) 106 (5) 19-22. Journal code: 0415216. ISSN: 0042-465X. Pub. country: USSR. Language: Russian.

AB Clinical picture of extraocular foreign bodies in the orbit is analyzed in 49 patients, specific features of the diagnosis and treatment of this condition are discussed. Study of the topography of foreign bodies in the orbit has helped distinguish the clinically significant area of fragment localization, areas at a high risk of developing complications, and 'quite' areas. A **localizing probe** on a space molding was found an effective diagnostic tool. Using this probe, stereotopic localization of a foreign body may be associated with roentgen-negative intraorbital structures, i.e. vessels, nerves, muscles. Topographic location of a foreign body helped explain a considerable vision acuity reduction in relatively transparent media, recurrences of hemorrhages to the fundus oculi, and the type of the pain syndrome. Foreign body removal is indicated in stubborn persistent pain and regressive time course of changes.

L2 ANSWER 6 OF 6 MEDLINE DUPLICATE 2

80223465 Document Number: 80223465. PubMed ID: 6156040. Fluorescent probes to detect lymphocyte activation. Nairn R C; Rolland J M. CLINICAL AND EXPERIMENTAL IMMUNOLOGY, (1980 Jan) 39 (1) 1-13. Ref: 157. Journal code: 0057202. ISSN: 0009-9104. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Fluorescent probes can monitor events in lymphocytes stimulated by mitogens and antigens. Early activation is associated with conformational changes in membrane macromolecules, and has been studied by measurement of fluorescence intensity or polarization of the membrane-**localizing probes** ANS, NPN, DPH and TMRITC. Subsequent changes in cytoplasmic macromolecules have been detected by altered fluorescence polarization of intracellular fluorescein. Altered metabolic activity in the activated lymphocyte is also revealed by fluorescent probes: the increased red fluorescence of lysosomes seen by AO staining, is attributable to altered lysosome membrane permeability. AO fluorescence has also detected early changes in the nuclear nucleoprotein complex. The later synthesis of new DNA is readily demonstrated by increased staining with the nuclear probes AO, ethidium bromide, propidium iodide, mithramycin and the Hoechst dyes. Adaptation of fluorescent probe analyses to the now rapidly developing flow microfluorimeters is providing rapid and sensitive assays of

lymphocyte stimulation. Such methods will permit routine detection of lymphocyte response to particular antigens or mitogens, as well as identification of antigenic substances by their stimulation of known reactive lymphocytes. Last but not least, fluorescent probes are providing new understanding of the cellular events and regulatory mechanisms associated with lymphocyte activation.

```
=> s cell transfection
   4 FILES SEARCHED...
L3         4684 CELL TRANSFECTION

=> s l3 and single chain antibody
L4            3 L3 AND SINGLE CHAIN ANTIBODY

=> s l4 and membrane bound
L5            0 L4 AND MEMBRANE BOUND

=> s single chain antibody
L6         4201 SINGLE CHAIN ANTIBODY

=> s l6 and membrane bound
L7           45 L6 AND MEMBRANE BOUND

=> s l7 and probe
L8            1 L7 AND PROBE

=> d l8 cbib abs

L8   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2003 ACS
2001:12605   Document No. 134:81775  Glycoprotein VI cDNA and protein from
     human and murine blood platelets and their diagnostic and therapeutic
     applications.  Busfield, Samantha J.; Villelal, Jean-luc; Jandrot-Perrus,
     Martine; Vainchencker, William; Gill, Davinder Singh; Qian, Ming Diana;
     Kingsbury, Gillian (Millennium Pharmaceuticals, Inc., USA).  PCT Int.
     Appl. WO 2001000810 A1 20010104, 227 pp.  DESIGNATED STATES: W:  AE, AG,
     AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK,
     DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
     KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
     NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,
     UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE,
     BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT,
     LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).  CODEN: PIXXD2.
     APPLICATION: WO 2000-US18152 20000630.  PRIORITY: US 1999-345468 19990630;
     US 1999-454824 19991206; US 2000-503387 20000214.
AB   The invention provides isolated cDNA mols. and polypeptide mols. that
     encode human and murine glycoprotein VI, a platelet membrane glycoprotein
     that is involved platelet-collagen interactions.  The protein initially
     designated TANGO 268 represents the platelet-expressed collagen receptor
     glycoprotein VI (GPVI) based on the following evidence: (1) the
     glycosylated mol. wts. of TANGO 268 and GPVI are identical or similar; (2)
     both are recognized by anti-GPVI antibodies and bind to convulxin; (3)
     both are preferentially expressed in megakaryocytic cells; (4) both ar
     predicted to have a single N-glycosylation site; (5) the mol. mass of GPVI
     upon N- and O-linked glycosylation is .apprx.62 kDa, that of GPVI; (6) two
     Ig-like domains in TANGO 268 indicates interaction with FcR.gamma.; (7)
     the absence of a large intracytoplasmic tail suggests that this
     membrane-bound glycoprotein has no signaling role but
     assocs. with another member of the Ig family; and (8) TANGO 268 has a
     charged arginine residue in the transmembrane domain which is also
     predicted to be present in GPVI.  The human gene for GPVI was mapped on
     radiation hybrid panels to the long arm of chromosome 19, in the region
     19q13, syntenic to mouse chromosome 7.  The invention also provides
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antisense nucleic acid mols., expression vectors contg. the nucleic acid mols. of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid mol. of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and antibodies. Diagnostic, screening and therapeutic methods utilizing compns. of the invention are also provided.

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L9 19 DUP REMOVE L7 (26 DUPLICATES REMOVED)

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L9 ANSWER 1 OF 19 CAPLUS COPYRIGHT 2003 ACS
2002:31529 Document No. 136:117377 Antibodies to B lymphocyte stimulator (BLyS). Ruben, Steven M.; Barash, Steven C.; Choi, Gil H.; Vaughan, Tristan; Hilbert, David (Human Genome Sciences, Inc., USA; Cambridge Antibody Technology Group Plc). PCT Int. Appl. WO 2002002641 A1 20020110, 3148 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US19110 20010615. PRIORITY: US 2000-PV212210 20000616; US 2000-PV240816 20001017; US 2001-PV276248 20010316; US 2001-PV277379 20010321; US 2001-PV293499 20010525.
AB The authors disclose the prepn. and characterization of single-chain antibodies that specifically bind to BLyS. The present invention also relates to methods and compns. for detecting, diagnosing, or treating a disease or disorder assocd. with aberrant BLyS expression.

L9 ANSWER 2 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2002:369099 Document No.: PREV200200369099. Cytotoxic T lymphocyte associated antigen-4 (CTLA-4) engagement has a long lasting effect on subsequent T cell responses. Engelhardt, John Joseph (1); Kuhns, Michael; Sullivan, Timothy; Allison, James P.. (1) Molecular and Cell Biology, University of California, 415 Life Science Addition, Berkeley, CA, 94720 USA. FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A711. http://www.fasebj.org/. print. Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002 ISSN: 0892-6638. Language: English.
AB CTLA-4 provides an inhibitory signal for T cell activation when interacting with its ligands, B7.1 and B7.2, on antigen presenting cells(APCs). This inhibitory signal can function in primary and previously activated T cells. We have found that engagement of CTLA-4 in primary stimulations can have long lasting effects upon T cell responses to subsequent antigen encounter. Artificial APCs that express appropriate MHC class II molecules and **membrane bound single chain antibodies** (scFvs) against CTLA-4 and CD28 were used to specifically ligate either CD28, CTLA-4 or both CD28 and CTLA-4 during primary stimulations of TCR transgenic cells with their cognate peptide antigen. Upon restimulation with peptide-MHC bearing APCs expressing B7.2, rather than scFvs, cells that had CTLA-4 ligated in the primary stimulation were less responsive in secondary stimulations, based on proliferation and IFN-g secretion. These differences corresponded with different protein tyrosine phosphorylation patterns seen before and after secondary antigen encounter. These data suggest that CTLA-4 ligation, upon primary antigen encounter, affects T cell responses to future antigen

encounter, and that there is a sustained biochemical basis for this effect.

L9 ANSWER 3 OF 19 MEDLINE DUPLICATE 1
2002363244 Document Number: 22104567. PubMed ID: 12109217. The recombinant T cell receptor strategy: insights into structure and function of recombinant immunoreceptors on the way towards an optimal receptor design for cellular immunotherapy. Hombach Andreas; Heuser Claudia; Abken Hinrich. (Lab. Tumorgenetik, Klinik I fur Innere Medizin, Universitat zu Koln, Joseph-Stelzmann-Str. 9, D-50924 Koln, Germany.. andreas.hombach@medizin.uni-koeln.de) . Curr Gene Ther, (2002 May) 2 (2) 211-26. Ref: 115. Journal code: 101125446. ISSN: 1566-5232. Pub. country: Netherlands. Language: English.

AB A promising approach in adoptive immunotherapy is based on the induction of a specific cellular anti-tumor response by antigen-specific, cytolytic T cells. Due to difficulties in isolating tumor-specific T cells in sufficient amounts, it was proposed to graft cytolytic T cells with an antigen-specific, recombinant T cell receptor. The antigen binding domain of the receptor consists of a **single-chain antibody** fragment (scFv) that is derived from a monoclonal antibody and binds to a tumor associated antigen. The intracellular signalling domain is derived from the cytoplasmic part of a **membrane bound** receptor to induce cellular activation, e.g., the Fc epsilon RI receptor gamma-chain or the CD3 zeta-chain. By use of this type of recombinant receptor, the strategy combines the advantages of MHC-independent, antibody-based antigen binding with efficient T cell activation upon specific binding to the receptor ligand. The modular composition of the receptor, moreover, facilitates modification of both the antigen binding and signalling properties. Accordingly, we and others have generated a panel of recombinant T cell receptors with specificities for malignantly or virally transformed cells. Receptor grafted effector cells were demonstrated to mediate a highly efficient immune response towards antigen expressing target cells. However, little is known about the impact of the recombinant receptor modules on recognition of highly heterologous target antigens and on cellular activation in a complex immunological context. This review summarizes the current knowledge about the generation and function of recombinant immunoreceptors and discusses the limitations and perspectives of the methodology for use in cellular immunotherapy.

L9 ANSWER 4 OF 19 MEDLINE DUPLICATE 2
2002126344 Document Number: 21850883. PubMed ID: 11861067. Characterization of a unique human **single-chain antibody** isolated by phage-display selection on **membrane -bound** mosquito midgut antigens. Foy Brian D; Killeen Gerry F; Frohn Ross H; Impoinvil Daniel; Williams Andrew; Beier John C. (Interdisciplinary Program of Molecular and Cellular Biology, Tulane University, New Orleans, LA, USA.. bfoy@tulane.edu) . JOURNAL OF IMMUNOLOGICAL METHODS, (2002 Mar 1) 261 (1-2) 73-83. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB The insect midgut is the primary site for food digestion, as well as for vector-borne pathogen infection into the invertebrate host. Accordingly, antigens of this critical insect organ are targets for anti-vector vaccines, insecticidal toxins, and transmission-blocking vaccines. We used midgut proteins of the African malaria vector mosquito Anopheles gambiae to select single-chain human antibody fragments (scFv) from a high-diversity, phage-displayed library. Using a phage-display selection method on western-blotted antigens, we selected an unusual truncated scFv clone, consisting of a heavy-chain only, which binds to An. gambiae midgut tissue. This clone binds a spectrum of mosquito antigens from the midgut and other mosquito tissues, as well as various mammalian glycoproteins, but binding was reduced when these glycoproteins were enzymatically deglycosylated. We also observed that this clone preferentially binds the

lumenal midgut surface. Furthermore, antigen binding by our selected scFv was limited by competition with increasing concentrations of certain soluble carbohydrates, most dramatically by galactose and N-acetyl glucosamine. Our results show that the cognate epitope of this scFv is a carbohydrate moiety. This paper describes a phage-display selection of antibody fragments on mosquito midgut tissue and it also describes a method for phage-display selection on membrane-immobilized heterogeneous antigens. These selection methods resulted in the isolation of a novel, truncated, carbohydrate-binding human antibody fragment from a naive phage-display library.

L9 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2003 ACS

2001:12605 Document No. 134:81775 Glycoprotein VI cDNA and protein from human and murine blood platelets and their diagnostic and therapeutic applications. Busfield, Samantha J.; Villelal, Jean-luc; Jandrot-Perrus, Martine; Vainchencker, William; Gill, Davinder Singh; Qian, Ming Diana; Kingsbury, Gillian (Millennium Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2001000810 A1 20010104, 227 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US18152 20000630. PRIORITY: US 1999-345468 19990630; US 1999-454824 19991206; US 2000-503387 20000214.

AB The invention provides isolated cDNA mols. and polypeptide mols. that encode human and murine glycoprotein VI, a platelet membrane glycoprotein that is involved platelet-collagen interactions. The protein initially designated TANGO 268 represents the platelet-expressed collagen receptor glycoprotein VI (GPVI) based on the following evidence: (1) the glycosylated mol. wts. of TANGO 268 and GPVI are identical or similar; (2) both are recognized by anti-GPVI antibodies and bind to convulxin; (3) both are preferentially expressed in megakaryocytic cells; (4) both ar predicted to have a single N-glycosylation site; (5) the mol. mass of GPVI upon N- and O-linked glycosylation is .apprx.62 kDa, that of GPVI; (6) two Ig-like domains in TANGO 268 indicates interaction with FcR.gamma.; (7) the absence of a large intracytoplasmic tail suggests that this **membrane-bound** glycoprotein has no signaling role but assocs. with another member of the Ig family; and (8) TANGO 268 has a charged arginine residue in the transmembrane domain which is also predicted to be present in GPVI. The human gene for GPVI was mapped on radiation hybrid panels to the long arm of chromosome 19, in the region 19q13, syntenic to mouse chromosome 7. The invention also provides antisense nucleic acid mols., expression vectors contg. the nucleic acid mols. of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid mol. of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and antibodies. Diagnostic, screening and therapeutic methods utilizing compns. of the invention are also provided.

L9 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2003 ACS

2001:129397 Document No. 134:324821 Development and applications of surface-linked **single chain antibodies** against T-cell antigens. Griffin, M. D.; Holman, P. O.; Tang, Q.; Ashourian, N.; Korthauer, U.; Kranz, D. M.; Bluestone, J. A. (Ben May Institute for Cancer Research, Dept. of Pathology and Committee on Immunology, University of Chicago, Chicago, IL, USA). Journal of Immunological Methods, 248(1-2), 77-90 (English) 2001. CODEN: JIMMBG. ISSN: 0022-1759. Publisher: Elsevier Science B.V..

AB A review with 57 refs. In this report the use of surface-linkage to

expand the potential exptl. and therapeutic applications of **single chain antibody** (scFv) constructs is reviewed. A strategy for the generation and functional characterization of surface-linked scFvs that bind selectively to the T-cell proteins CD3.epsilon., CD28, and CD152 (CTLA-4) is described in detail. Exptl. examples are provided of the use of these constructs to study the pos. and neg. regulation of T-cell activation and to manipulate the in vivo immunogenicity of tumor cells. In addn., a novel system for Simultaneous T-cell Activation and Retroviral Transduction (START) is described in which retroviral packaging cells are rendered mitogenic for T lymphocytes by combined expression of surface-linked scFvs. Finally, the use of random mutagenesis and yeast surface display to increase the affinity and functional efficacy of scFv constructs is demonstrated.

L9 ANSWER 7 OF 19 CAPLUS COPYRIGHT 2003 ACS

2001:180188 Document No. 134:324940 Selection of single-chain Fv antibodies to colorectal cancer antigens and preliminary characterization. Zhu, Jian-gao; Hu, Jing-yue; Li, Guan-cheng; Li, Yue-hui; Zhou, Guo-hua; Li, Xiao-ling; Sun, Qu-bing (Cancer Research Inst., Hunan Med. Univ., Changsha, 410078, Peop. Rep. China). Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao, 17(1), 18-22 (Chinese) 2001. CODEN: ZSHXF2. ISSN: 1007-7626. Publisher: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui.

AB To isolate tumor-specific phage antibodies from single-chain Fv fusion phage library that was constructed from B-lymphocytes of patients with colorectal cancer, selections of phage antibody library were performed on complex antigen sources such as whole live cells or tissue sections, or even by in vivo selection methods. First, the library was subjected to three rounds of pos. selection on human colorectal cancer cells and two rounds of neg. selection on human fibroblast cells and peripheral blood mononuclear cells (PBMC). Phages that bound cell membrane and that were internalized into cells were recovered. Enrichment of **membrane-bound** and internalized phages was 430 times. Second, in vivo selections were performed in LS174T/HRT-18-xenografted mice. After phage library was injected into nude mice, specific phages were retrieved from tumor tissues at the variable time point of the expts. The efficiency of phage recovery was the best at the time point of 24 h. Finally, for selection on tissue sections, phages recovered on tissue cryosections were 1.6 times higher than those on tissue paraffin pos. sections. Clones of phage antibodies were tested by cell ELISA, and 5 anti-colorectal cancer phage antibody clones were isolated. Clones of 4 of 5 ScFv-phages demonstrated specific tumor reactivity to 4 different colorectal cancer cell lines but not to 7 other tumor cell lines such as lung, liver, ovarian, nasopharyngeal and cervical cancer, and one reacted to all cancer cell lines, specifically. Three selection approaches used were suitable for selection of anti-tumor phage antibodies.

L9 ANSWER 8 OF 19 MEDLINE DUPLICATE 3

2000243538 Document Number: 20243538. PubMed ID: 10779742. Blockade of T cell activation using a surface-linked **single-chain antibody** to CTLA-4 (CD152). Griffin M D; Hong D K; Holman P O; Lee K M; Whitters M J; O'Herrin S M; Fallarino F; Collins M; Segal D M; Gajewski T F; Kranz D M; Bluestone J A. (The Ben May Institute for Cancer Research and Department of Pathology and Committee on Immunology, University of Chicago, Chicago, IL 60637, USA.) JOURNAL OF IMMUNOLOGY, (2000 May 1) 164 (9) 4433-42. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB CTLA-4 (CD152) engagement can down-regulate T cell activation and promote the induction of immune tolerance. However, the strategy of attenuating T cell activation by engaging CTLA-4 has been limited by sharing of its natural ligands with the costimulatory protein CD28. In the present study, a CTLA-4-specific single-chain Ab (scFv) was developed and expressed on the cell surface to promote selective engagement of this regulatory

molecule. Transfectants expressing anti-CTLA-4 scFv at their surface bound soluble CTLA-4 but not soluble CD28. Coexpression of anti-CTLA-4 scFv with anti-CD3epsilon and anti-CD28 scFvs on artificial APCs reduced the proliferation and IL-2 production by resting and preactivated bulk T cells as well as CD4+ and CD8+ T cell subsets. Importantly, expression of anti-CTLA-4 scFv on the same cell surface as the TCR ligand was essential for the inhibitory effects of CTLA-4-specific ligation. CTLA-4-mediated inhibition of tyrosine phosphorylation of components of the proximal TCR signaling apparatus was similarly dependent on coexpression of TCR and CTLA-4 ligands on the same surface. These findings support a predominant role for CTLA-4 function in the modification of the proximal TCR signal. Using T cells from DO11.10 and 2C TCR transgenic mice, negative regulatory effects of selective CTLA-4 ligation were also demonstrated during the stimulation of Ag-specific CD4+ and CD8+ T cells by MHC/peptide complexes. Together these studies demonstrate that selective ligation of CTLA-4 using a **membrane-bound** scFv results in attenuated T cell responses only when coengaged with the TCR during T cell/APC interaction and define an approach to harnessing the immunomodulatory potential of CTLA-4-specific ligation.

L9 ANSWER 9 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2001:210590 Document No.: PREV200100210590. Growth retardation of a human colon carcinoma in SCID mice after vaccination with tumor cells expressing **membrane-bound single-chain antibodies**. Diaz, C. de Ines (1); Cochlovius, B. (1); Choi, I. (1); Little, M.. (1) Recombinant Antibody Research Group, DKFZ, Heidelberg Germany. Immunobiology, (November, 2000) Vol. 203, No. 1-2, pp. 353. print. Meeting Info.: Joint Annual Meeting of the German and Dutch Societies of Immunology Duseldorf, Germany November 29-December 02, 2000 ISSN: 0171-2985. Language: English. Summary Language: English.

L9 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2003 ACS
1999:595196 Document No. 131:195457 Simian virus 5 (SV5) vector which encodes a foreign protein in place of the HN glycoprotein, and uses thereof in delivering recombinant molecules to specific cells. Parks, Griffith D. (Wake Forest University, USA). PCT Int. Appl. WO 9946278 A1 19990916, 27 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US5388 19990312. PRIORITY: US 1998-41987 19980313.

AB The present invention provides viral vectors derived from the paramyxovirus, simian virus 5 (SV5) and discloses uses thereof for the delivery of recombinant mols. to specific cells. SV5 contains two viral glycoproteins in its envelope: the HN protein which functions in attachment to host cell receptors, and the F protein which fuses the virion envelope with the target cell plasma membrane. In this invention, SV5 is engineered to encode a foreign protein in place of HN, thereby allowing for the targeting of the viral vector to cells expressing the ligand that is complementary to said foreign virion-assocd. protein or glycoprotein. In one embodiment of the invention, rSV5 is engineered to incorporate into its envelope a **membrane-bound** form of a **single chain antibody** (sFv) which is specific for antigen HER2, an antigen that is overexpressed in a large no. of carcinomas. Thus, the invention relates to a novel gene delivery system that provides a simple and flexible method for overcoming the limitation in the prior art of specifically targeting infection of a recombinant virus to a predetd. population of cells.

L9 ANSWER 11 OF 19 MEDLINE DUPLICATE 4
1999421849 Document Number: 99421849. PubMed ID: 10490996. Apoptosis of a human melanoma cell line specifically induced by **membrane-bound single-chain antibodies**. de Ines C; Cochlovius B; Schmidt S; Kipriyanov S; Rode H J; Little M. (Recombinant Antibody Group, Department of Tumor Progression and Immune Defense, Experimental Therapy and Diagnosis Programme, German Cancer Research Center, Heidelberg.) JOURNAL OF IMMUNOLOGY, (1999 Oct 1) 163 (7) 3948-56. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB CD28 is a key regulatory molecule in T cell responses. Ag-TCR/CD3 interactions without costimulatory signals provided by the binding of B7 ligands to the CD28R appear to be inadequate for an effective T cell activation. Indeed, the absence of B7 on the tumor cell surface is probably one of the factors contributing to the escape of tumors from immunological control and destruction. Therefore, to increase the immunogenicity of tumor cell vaccines, we have expressed anti-CD3 and anti-CD28 single-chain Abs (scFv) separately on the surface of a human melanoma SkMel63 cell line (HLA-A*0201). A mixture of cells expressing anti-CD3 with cells expressing anti-CD28 resulted in a marked activation of allogeneic human PBL in vitro. The apparent induction of a Th1 differentiation pathway was accompanied by the proliferation of MHC-independent NK cells and MHC-dependent CD8+ T cells. PBL that had been cultured together with transfected SkMel63 tumor cells were able to specifically induce apoptosis in untransfected SkMel63 cells. In contrast, three other tumor cell lines expressing HLA-A*0201, including two melanoma cell lines, showed no significant apoptosis. These results provide valuable information for both adoptive immunotherapy and the generation of autologous tumor vaccines.

L9 ANSWER 12 OF 19 MEDLINE DUPLICATE 5
1999363973 Document Number: 99363973. PubMed ID: 10435115. A chimeric receptor that selectively targets **membrane-bound** carcinoembryonic antigen (mCEA) in the presence of soluble CEA. Hombach A; Koch D; Sircar R; Heuser C; Diehl V; Kruis W; Pohl C; Abken H. (Klinik I fur Innere Medizin, Labor fur Tumorgenetik, Universitat zu Koln, Germany.) GENE THERAPY, (1999 Feb) 6 (2) 300-4. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Chimeric T cell receptors with specificity for tumor-associated antigens are successfully used to target T cells to tumor cells. The efficacy of this approach, however, is reduced by soluble antigen that is frequently present in high serum concentrations. To overcome this situation, we constructed an anti-CEA chimeric receptor whose extracellular moiety is composed of a humanized **single chain antibody** fragment (scFv) derived from the anti-CEA mAb BW431/26 and the CH2/CH3 constant domains of human IgG. The intracellular moiety consists of the gamma-signaling chain of the human Fc epsilon RI receptor constituting a completely humanized chimeric receptor. After transfection, the humBW431/26 scFv-CH2CH3-gamma receptor is expressed as a homodimer on the surface of MD45 T cells. Co-incubation with CEA+ tumor cells specifically activates grafted MD45 T cells indicated by IL-2 secretion and cytolytic activity against CEA+ tumor cells. Notably, the efficacy of receptor-mediated activation is not affected by soluble CEA up to 25 micrograms/ml demonstrating the usefulness of this chimeric receptor for specific cellular activation by **membrane-bound** CEA even in the presence of high concentrations of CEA, as found in patients during progression of the disease.

L9 ANSWER 13 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1999:87718 Document No.: PREV199900087718. Isolation of **single chain antibody** fragments (SCFV) with specificity to cell surface antigens by phage display utilizing internal image anti-idiotypic antibodies. Hombach, Andreas (1); Pohl, Christoph; Heuser, Claudia (1);

Sircar, Ranjan (1); Diehl, Volker (1); Abken, Hinrich (1). (1) Klinik I Innere Medizin, Labor Tumorgenetik, Univ. Koeln, Josef-Stelzmann-Str. 9, D-50924 Koeln Germany. Annals of Hematology, (1998) Vol. 77, No. SUPPL. 2, pp. S81. Meeting Info.: Annual Congress of the German and Austrian Societies of Hematology and Oncology Frankfurt, Germany October 25-28, 1998 Austrian Society of Hematology and Oncology. ISSN: 0939-5555. Language: English.

L9 ANSWER 14 OF 19 MEDLINE DUPLICATE 6
1999034431 Document Number: 99034431. PubMed ID: 9819122. Isolation of **single chain antibody** fragments with specificity for cell surface antigens by phage display utilizing internal image anti-idiotypic antibodies. Hombach A; Pohl C; Heuser C; Sircar R; Diehl V; Abken H. (Klinik I fur Innere Medizin, Labor Tumorgenetik, Universitat zu Koln, Germany.) JOURNAL OF IMMUNOLOGICAL METHODS, (1998 Sep 1) 218 (1-2) 53-61. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Recombinant **single chain antibody** fragments (scFv) with specificity for **membrane-bound** antigens can be isolated by phage display techniques. The strategy involves selection of recombinant phage antibodies by binding to cells expressing the respective antigen. This results frequently in high nonspecific adherence of phages to cellular membranes. To resolve the problem we have made use of an internal image anti-idiotypic antibody mimicking the **membrane-bound** CD30 antigen and successfully isolated scFv fragments with specificity for CD30. The cDNA coding for the immunoglobulin heavy and light chain variable regions of the anti-CD30 monoclonal antibody (mAb) HRS3 was expressed by phage display techniques. Recombinant HRS3-scFv phages were efficiently enriched by one cycle of panning on the internal image anti-idiotypic mAb 9G10. The isolated HRS3-scFv clone retained the binding specificity of the parental mAb HRS3 to the internal image anti-idiotypic mAb 9G10 as well as to an anti-idiotypic mAb without the internal image. Furthermore HRS3-scFv reacted with recombinant and cell-bound CD30 antigen, respectively. Binding of scFv fragments to anti-idiotypic mAbs will provide a versatile strategy for the efficient isolation of recombinant antibody fragments.

L9 ANSWER 15 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1998:511427 Document No.: PREV199800511427. Isolation of **single chain antibody** fragments with specificity for cell surface antigens by phage display utilizing internal image anti-idiotypic antibodies. Hombach, Andreas; Pohl, Christoph; Heuser, Claudia; Sircar, Ranjan; Diehl, Volker; Abken, Hinrich (1). (1) Klin. I Innere Med., Labor Tumorgenetik, Univ. Koeln, Josef-Stelzmann-Str. 9, D-50924 Koeln Germany. Journal of Immunological Methods, (Sept. 1, 1998) Vol. 21, No. 1-2, pp. 53-61. ISSN: 0022-1759. Language: English.

AB Recombinant **single chain antibody** fragments (scFv) with specificity for **membrane-bound** antigens can be isolated by phage display techniques. The strategy involves selection of recombinant phage antibodies by binding to cells expressing the respective antigen. This results frequently in high nonspecific adherence of phages to cellular membranes. To resolve the problem we have made use of an internal image anti-idiotypic antibody mimicking the **membrane-bound** CD30 antigen and successfully isolated scFv fragments with specificity for CD30. The cDNA coding for the immunoglobulin heavy and light chain variable regions of the anti-CD30 monoclonal antibody (mAb) HRS3 was expressed by phage display techniques. Recombinant HRS3-scFv phages were efficiently enriched by one cycle of panning on the internal image anti-idiotypic mAb 9G10. The isolated HRS3-scFv clone retained the binding specificity of the parental mAb HRS3 to the internal image anti-idiotypic mAb 9G10 as well as to an anti-idiotypic mAb without the internal image. Furthermore HRS3-scFv reacted with recombinant and cell-bound CD30 antigen, respectively.

Binding of scFv fragments to anti-idiotypic mAbs will provide a versatile strategy for the efficient isolation of recombinant antibody fragments.

L9 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2003 ACS

1997:283740 Document No. 126:260128 Rapid affinity selection of transformed eukaryotic cells using immunoglobulins as affinity labels. Chesnut, Robert D.; Baytan, Apollo; Hoeffler, James P.; Bernhard, Amy; Chang, Mei-ping (Invitrogen Corporation, USA). PCT Int. Appl. WO 9708186 A1 19970306, 81 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US15819 19960823. PRIORITY: US 1995-518835 19950824.

AB A novel expression system that allows the study of exptl. genes of interest soon after transfection is described. The method includes a vector that encodes an antibody deriv. that can be used as an affinity label. The antibody is to a convenient hapten and is modified by inclusion of a signal peptide and transmembrane domain to direct and bind the antibody to the cell membrane. This allows identification and selection of transfected cells from culture to be carried out immediately, within hours, after the transfection event. The invention also relates to cells transfected with the expression system and methods for selection and isolation of cells transfected with the expression system. The development of a system using a **single-chain antibody** to oxazolone is described. The antibody uses the transmembrane domain of a platelet-derived growth factor receptor and the mouse Ig .kappa. chain V-J2-C region signal peptide to direct the antibody into the cell membrane. Myc.1 and hemagglutinin epitope tags were included at the N- and C-termini to assay for complete and accurate synthesis of the protein. Selection of transfected cells by binding to magnetic particles coated with BSA conjugates with oxazole is demonstrated. Selection was possible within 8-24 h post transfection. Simultaneous transformation with this vector and a reporter gene vector resulted in 98% of the affinity-selected cells expressing the reporter gene.

L9 ANSWER 17 OF 19 MEDLINE DUPLICATE 7

95001873 Document Number: 95001873. PubMed ID: 7918382. Functional immunoliposomes harboring a biosynthetically lipid-tagged **single -chain antibody**. Laukkanen M L; Alfthan K; Keinanen K. (VTT Biotechnology and Food Research, Espoo, Finland.) BIOCHEMISTRY, (1994 Sep 27) 33 (38) 11664-70. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB An anti-2-phenyloxazolone **single-chain antibody** was expressed in Escherichia coli as a lipoprotein fusion in order to generate a biosynthetically lipid-tagged molecule [Laukkanen et al. (1993) Protein Eng. 6, 449-454]. For purification, a hexahistidinyl tag was introduced to the C-terminus of the protein. The resulting antibody, termed Ox lpp-scFv-H6, was **membrane-bound**, displayed hapten-binding activity, and contained the lipoprotein-specific lipid modification as indicated by metabolic [3H]palmitic acid labeling. The Ox lpp-scFv-H6 was purified by immobilized metal affinity chromatography followed by hapten-based affinity chromatography to essential homogeneity with a yield of 0.4-1.6 mg/L of culture. In detergent dialysis, the purified antibody partitioned quantitatively into phospholipid liposomes. The immunoliposome preparation consisting of a homogeneous population of unilamellar 100-200 nm vesicles displayed specific hapten-binding activity as measured by using ELISA and surface plasmon resonance (SPR)-based real-time biospecific interaction analysis. In SPR experiments, the immunoliposomes exhibited virtually irreversible

binding to immobilized hapten compared to soluble antibody fragments, consistent with the predicted multivalent binding. Biosynthetic lipid-tagging of antibodies may prove useful for immunoliposome-based diagnostic and therapeutic applications.

L9 ANSWER 18 OF 19 SCISEARCH COPYRIGHT 2003 ISI (R)
94:371181 The Genuine Article (R) Number: NQ569. BIOSYNTHETIC LIPID-TAGGING OF ANTIBODIES. KEINANEN K (Reprint); LAUKKANEN M L. VTT, BIOTECHNOL & FOOD RES, POB 1500, SF-02044 ESPOO, FINLAND (Reprint). FEBS LETTERS (06 JUN 1994) Vol. 346, No. 1, pp. 123-126. ISSN: 0014-5793. Pub. country: FINLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Chemical conjugation of fatty acids to antibodies generates lipid-modified molecules which have found use in the targeting of liposome-mediated drug delivery and in liposome-based immunoassays. Alternatively, bacterial expression of antibodies as single-chain Fv fragments fused to lipoprotein signal peptide and N-terminal sequence leads to in vivo enzymatic addition of a single glycerolipid group at the N-terminus of the molecule. This lipid-modification converts the antibody from a soluble protein into a functional **membrane-bound** molecule. These biosynthetically lipid-tagged antibodies may prove useful for immobilization of antibodies to membranes in various biotechnological applications.

L9 ANSWER 19 OF 19 MEDLINE DUPLICATE 8
93324525 Document Number: 93324525. PubMed ID: 8332603. Lipid-tagged antibodies: bacterial expression and characterization of a lipoprotein-**single-chain antibody** fusion protein.
Laukkanen M L; Teeri T T; Keinanen K. (VTT Biotechnical Laboratory, Espoo, Finland.) PROTEIN ENGINEERING, (1993 Jun) 6 (4) 449-54. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.
AB In order to achieve a stable and functional immobilization of antibodies, we investigated the possibility of adding hydrophobic membrane anchors to antibody fragments expressed in Escherichia coli. The DNA sequence encoding the signal peptide and the nine N-terminal amino acid residues of the major lipoprotein of E.coli was fused to the sequence of an anti-2-phenyloxazolone single-chain Fv antibody fragment [Takkinen et al. (1991) Protein Engng, 4,837-841]. The expression of the fusion construct in E.coli resulted in specific accumulation of an immunoreactive 28 kDa polypeptide. Unlike the unmodified single-chain Fv fragment, the fusion protein was cell-associated, labelled by [3H]palmitate which is indicative of the presence of N-terminal lipid modification, partitioned into the detergent phase upon Triton X-114 phase separation and was localized predominantly in the bacterial outer membrane. The fusion antibody displayed specific 2-phenyloxazolone-binding activity in the **membrane-bound** form and after solubilization with non-ionic detergents. Furthermore, upon removal of detergent the fusion antibody was incorporated into proteoliposomes which displayed specific hapten-binding activity. Our results show that antibodies can be converted to **membrane-bound** proteins with retention of antigen-binding properties by introduction of lipid anchors during biosynthesis. This approach may prove useful in the design of immunoliposomes and immunosensors.

=> s conjugate probe
L10 99 CONJUGATE PROBE

=> s l10 and membrane permeant
L11 0 L10 AND MEMBRANE PERMEANT

=> s l10 and bind sv

L12 0 L10 AND BIND SV

=> s l10 and PhOx
L13 0 L10 AND PHOX

=> s "PhOx" conjugate
L14 5 "PHOX" CONJUGATE

=> dup remove l14
PROCESSING COMPLETED FOR L14
L15 1 DUP REMOVE L14 (4 DUPLICATES REMOVED)

=> d l15 cbib abs

L15 ANSWER 1 OF 1 MEDLINE DUPLICATE 1
2001515560 Document Number: 21230639. PubMed ID: 11333112. Evaluation of the process for superoxide production by NADPH oxidase in human neutrophils: evidence for cytoplasmic origin of superoxide. Kobayashi T; Tsunawaki S; Seguchi H. (Department of Anatomy and Cell Biology, Kochi Medical School, Japan.) REDOX REPORT, (2001) 6 (1) 27-36. Ref: 75. Journal code: 9511366. ISSN: 1351-0002. Pub. country: England: United Kingdom. Language: English.

AB We present an up-to-date insight into the function of NADPH oxidase in human neutrophils, the signalling pathways involved in activation of this enzyme and the process of association of its components with the cytoskeleton. We also discuss the functional implications of morphological studies revealing localization of the sites of NADPH oxidase activity. An original model of the process of superoxide (O2*-) production in human neutrophils is shown. Organization of NADPH oxidase is associated with several components. Upon stimulation, tri-phox cytosolic components of NADPH oxidase (p40-phox, p47-phox and p67-phox) bind to actin filaments. This process involves other actin-binding proteins, such as cofilin and coronin. Activated protein kinase C, translocated from the plasma membrane, phosphorylates cytosolic components at a scaffold of cytoskeleton. Subsequently, p40-phox, responsible for maintaining the resting state of NADPH oxidase, is separated from other two cytosolic phox proteins following an attachment of the active form of small GTP-binding protein Rac to p67-phox. Cytosolic duo-phox proteins (p47-phox and p67-**phox**) **conjugate** with membrane components (gp91-phox, p22-phox and Rapla) of NADPH oxidase residing within membranes of intracellular compartments. This chain of events triggers production of O2*-. Then, oxidant-producing intracellular compartments associate with the plasma membrane. Eventually, intracellularly produced O2*- is released to the extracellular environment through the orifice formed by fusion of oxidant-producing compartments with the plasma membrane. Intracellular movement of the oxidant-producing compartments may be regulated by myosin light chain kinase. The review emphasizes that functional assembly of NADPH oxidase and, therefore, generation of O2*- is accomplished essentially within the intracellular compartments. Upon neutrophil stimulation, intracellularly generated O2*- is transported to the plasma membrane to be released and to ensure host defense against infection.

=> s membrane permeant probe
L16 27 MEMBRANE PERMEANT PROBE

=> s l16 and conjugate
L17 0 L16 AND CONJUGATE

=> s l16 and linker
L18 0 L16 AND LINKER

=> dup remove l16

PROCESSING COMPLETED FOR L16
L19 9 DUP REMOVE L16 (18 DUPLICATES REMOVED)

=> d l19 1-9 cbib abs

L19 ANSWER 1 OF 9 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 1
1999276541 EMBASE Indium-111 CDTA-(aminostyryl)pyridinium (ASP) dyes as new radioactive cell **membrane permeant probes**:
Synthesis, leukocyte labeling, and serum stability. Mease R.C.; Lambert C.; McAfee J.G.. R.C. Mease, Department of Diagnostic Radiology, University of Maryland, Baltimore, Baltimore, MD 21201, United States.
Journal of Labelled Compounds and Radiopharmaceuticals 42/SUPPL. 1 (S815-S817) 1999.
Refs: 6.
ISSN: 0362-4803. CODEN: JLCRD4. Pub. Country: United Kingdom. Language: English.

L19 ANSWER 2 OF 9 MEDLINE DUPLICATE 2
2000097184 Document Number: 20097184. PubMed ID: 10631609. The chemical cell biology of zinc: structure and intracellular fluorescence of a zinc-quinolinesulfonamide complex. Nasir M S; Fahrni C J; Suhy D A; Kolodsick K J; Singer C P; O'Halloran T V. (Department of Chemistry, Northwestern University, Evanston, IL 60208-3113, USA.) JOURNAL OF BIOLOGICAL INORGANIC CHEMISTRY, (1999 Dec) 4 (6) 775-83. Journal code: 9616326. ISSN: 0949-8257. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB Fluorescent cell-permeant compounds based on 6-methoxy-8-p-toluenesulfonamido-quinoline, TSQ, are potentially powerful probes of intracellular zinc chemistry; however, the structure, thermodynamics, and stoichiometry of the metal complexes, and the molecular basis of Zn(II) recognition, remain open issues. To address these, we report the first structural characterization of a Zn(II) complex of a TSQ derivative, namely 2-methyl-6-methoxy-8-p-toluenesulfonamido-quinoline (3) and describe its unusual coordination chemistry. The crystal structure of the fluorescent complex of 3 with zinc reveals a 2:1 stoichiometry wherein bidentate coordination of two nitrogens from each ligand gives rise to a highly distorted tetrahedral Zn(II) center. Both sulfonamido groups in the zinc complex are tilted away from zinc to make room for coordination of the amide nitrogens. Zn-O(2) and Zn-O(4) distances are essentially nonbonding (3.06 and 3.10 A, respectively). The bond angles [N(1)-Zn-N(2) 83.5 degrees and N(3)-Zn-N(4) 83.0 degrees] are quite small relative to the 109 degrees angle of an ideal tetrahedral center. This result provides an insight into the zinc-binding mode of the TSQ derivative zinquin, in which a methyl group replaces the hydrogen in the 2-position of the quinoline ring. The methyl group and sulfonamide oxygen atoms clearly hinder formation of both square planar and octahedral complexes. We also show here that the Zn(II) complex of 3 in DMSO-water (80/20 w/w) exhibits an overall binding stability (log beta 2 = 18.24 +/- 0.02) similar to zinquin. Fluorescence microscopy suggests that each of these members of this family demarks a similar set of Zn(II)-enriched compartments that are common to all eukaryotic cells examined to date, and further shows that the ester function is not required for observation of these ubiquitous Zn-loaded compartments. The combined structural, thermodynamic, and physiological results provide a basis for design of other Zn(II)-specific **membrane permeant probes** with a range of
Zn(II) affinities and photophysical properties.

L19 ANSWER 3 OF 9 MEDLINE DUPLICATE 3
97153137 Document Number: 97153137. PubMed ID: 8999858. Histidine 225, a residue of the NhaA-Na+/H+ antiporter of Escherichia coli is exposed and faces the cell exterior. Olami Y; Rimon A; Gerchman Y; Rothman A; Padan E. (Division of Microbial and Molecular Ecology, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) JOURNAL OF BIOLOGICAL CHEMISTRY,

(1997 Jan 17) 272 (3) 1761-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cysteine residues were found nonessential in the mechanism of the NhaA antiporter activity of Escherichia coli. The functional C-less NhaA has provided the groundwork to study further histidine 225 of NhaA which has previously been suggested to play an important role in the activation of NhaA at alkaline pH (Rimon, A., Gerchman, Y., Olami, Y., Schuldiner, S. and Padan, E. (1995) J. Biol. Chem. 270, 26813-26817). C-less H225C was constructed and shown to possess an antiporter activity 60% of that of C-less antiporter and a pH profile similar to that of both the C-less or wild-type antiporters. Remarkably, whereas neither the wild-type nor the C-less antiporters were affected by N-ethylmaleimide, C-less H225C was inhibited by this reagent. To determine the degree of alkylation of the antiporter protein by N-ethylmaleimide, antiporter derivatives tagged at their C termini with six histidines residues were constructed. Alkylation of C-less H225C was measured by labeling of everted membrane vesicles with [14C]N-ethylmaleimide, affinity purification of the His-tagged antiporter, and determination of the radioactivity of the purified protein. This assay showed that H225C is alkylated to a much higher level than any of the native cysteinyl residues of NhaA reaching saturation at alkyl/NhaA stoichiometry of 1. The wild-type derivative showed at least 10-fold less alkylation even at higher concentrations, suggesting that H225C resides in a domain that is much more exposed to N-ethylmaleimide than the native cysteinyl residues of NhaA. Since H225C residues both in right-side out and inside-out membrane vesicles were quantitatively alkylated by N-ethylmaleimide, this assay was used to determine the accessibility of H225C to other SH reagents by titrating the H225C left free to react with N-ethylmaleimide, following exposure of the membranes to the reagents. Furthermore, since membrane-impermeant probes can react with residues in membrane-embedded protein only if accessible to the medium containing the reagent, the assay was used to determine the membrane topology of H225C. As expected for a **membrane-permeant probe**, p-chloromercuribenzoate reacted with H225C as efficiently as N-ethylmaleimide in both membrane orientations. Similar results were obtained with methanethiosulfonate ethylammonium supporting the recent observations that this probe is membrane-permeant. On the other hand, both membrane-impermeant reagents p-chloromercuribenzosulfonate and methanethiosulfonate ethyl-trimethyl ammonium bromide reacted with H225C 10-fold more in right-side out than in inside-out vesicles, and p-chloromercuribenzosulfonate also blocked completely the H225C in intact cells. These results strongly suggest that H225C is exposed at the periplasmic face of the membrane.

L19 ANSWER 4 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

1996:98415 Document No.: PREV199698670550. (Aminostyryl)pyridinium (ASP) dyes as a new radioactive cell **membrane permeant probes**: Synthesis of tributyltin precursors, radioiodination, development of mixed leukocyte and lymphocyte labeling procedures, and preliminary imaging studies in dogs with an induced inflammatory abscess. Lambert, C. (1); Mease, R. C.; Avren, L.; Le, T.; Sabel, H.; McAfee, J. G.. (1) George Washington University Medical Center, Washington, DC USA. Journal of Labelled Compounds and Radiopharmaceuticals, (1995) Vol. 37, No. 0, pp. 767-769. Meeting Info.: Eleventh International Symposium on Radiopharmaceutical Chemistry Vancouver, British Columbia, Canada August 13-17, 1995 ISSN: 0362-4803. Language: English.

L19 ANSWER 5 OF 9 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 4

94009149 EMBASE Document No.: 1994009149. Role of sodium ions for sulfate transport and energy metabolism in Desulfovibrio salexigens. Kreke B.; Cypiomka H.. Institut fur Chemie/Biol. des Meeres, Universitat Oldenburg, Postfach 2503,D-26111 Oldenburg, Germany. Archives of Microbiology 161/1 (55-61) 1994.
ISSN: 0302-8933. CODEN: AMICCW. Pub. Country: Germany. Language: English.

Summary Language: English.

AB The sodium ion gradient and the membrane potential were found to be the driving forces of sulfate accumulation in the marine sulfate reducer Desulfovibrio salexigens. The protonmotive force of -158 mV, determined by means of radiolabelled **membrane-permeant probes**, consisted of a membrane potential of -140 mV and a pH gradient (inside alkaline) of 0.3 at neutral pH (out). The sodium ion gradient, as measured with silicone oil centrifugation and atomic absorption spectroscopy, was eightfold ([Na+](out)/[Na+](in)) at an external Na+ concentration of 320 mM. The resulting sodium ion-motive force was -194 mV and enabled D. salexigens to accumulate sulfate 20000-fold at low external sulfate concentrations (< 0.1 .mu.M). Under these conditions high sulfate accumulation occurred electrogenically in symport with three sodium ions (assuming equilibrium with the sodium ion-motive force). With increasing external sulfate concentrations sulfate accumulation decreased sharply, and a second, low-accumulating system symported sulfate electroneutrally with two sodium ions. The sodium-ion gradient was built up by electrogenic Na+/H+ antiport. This was demonstrated by (i) measuring proton translocation upon sodium ion pulses, (ii) studying uptake of sodium salts in the presence or absence of the electrical membrane potential, and (iii) the inhibitory effect of the Na+/H+ antiport inhibitor propylbenzilylcholin-mustard HCl (PrBCM). With resting cells ATP synthesis was found after proton pulses (changing the pH by three units), but neither after pulses of 500 mM sodium ions, nor in the presence of the uncoupler tetrachorosalicylanilide (TCS). It is concluded that the energy metabolism of the marine strain D. salexigens is based primarily on the protonmotive force and a proton-translocating ATPase.

L19 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

1993:68626 Document No.: PREV199344034276. Quantitation of intracellular oxidation induced by hydrogen peroxide and bleomycin. Bhat, M.; Ma, J. K. H.; Weber, S.; Rojanasakul, Y.. Sch. Pharm., West Virginia Univ., Morgantown, W. Va. 26506. Pharmaceutical Research (New York), (1992) Vol. 9, No. 10 SUPPL., pp. S196. Meeting Info.: American Association of Pharmaceutical Scientists 1992 Annual Meeting and Exposition San Antonio, Texas, USA November 15-19, 1992 ISSN: 0724-8741. Language: English.

L19 ANSWER 7 OF 9 MEDLINE DUPLICATE 5

93074272 Document Number: 93074272. PubMed ID: 1332637. Protonmotive force in freshwater sulfate-reducing bacteria, and its role in sulfate accumulation in Desulfobulbus propionicus. Kreke B; Cypionka H. (Fakultat fur Biologie, Universitat Konstanz, Federal Republic of Germany.) ARCHIVES OF MICROBIOLOGY, (1992) 158 (3) 183-7. Journal code: 0410427. ISSN: 0302-8933. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The protonmotive force in several sulfate-reducing bacteria has been determined by means of radiolabelled **membrane-permeant probes** (tetraphenylphosphonium cation, TPP+, for delta psi, and benzoate for delta pH). In six of ten freshwater strains tested only the pH gradient could be determined, while the membrane potential was not accessible due to nonspecific binding of TPP+. The protonmotive force of the other four strains was between -110 and -155 mV, composed of a membrane potential of -80 to -140 mV and a pH gradient between 0.25 and 0.8 (inside alkaline) at pH(out) = 7. In Desulfobulbus propionicus the pH gradient decreased with rising external pH values. This decrease, however, was compensated by an increasing membrane potential. Sulfate, which can be highly accumulated by the cells, did not affect the protonmotive force, if added in concentrations of up to 4 mM. The highest sulfate accumulation observed (2500-fold), which occurred at external sulfate concentrations below 5 microM, could be explained by a symport of three protons per sulfate, if equilibrium with the protonmotive force was assumed. At higher sulfate concentrations the accumulation decreased and suggested an

electroneutral symport of two protons per sulfate. At sulfate concentrations above 500 microM, the cells stopped sulfate uptake before reaching an equilibrium with the protonmotive force.

L19 ANSWER 8 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
1980:151223 Document No.: BA69:26219. EFFECTS OF LIPOPHILIC AND WATER SOLUBLE MEMBRANE PROBES ON ETHYLENE SYNTHESIS IN APPLE AND PENICILLIUM-DIGITATUM. MATTOO A K; CHALUTZ E; LIEBERMAN M. DEP. PLANT GENET., WEIZMANN INST. SCI., REHOVOT, ISR.. PLANT CELL PHYSIOL, (1979) 20 (6), 1097-1106. CODEN: PCPHA5. ISSN: 0032-0781. Language: English.

AB Several chemicals were used to probe the in situ ethylene forming enzyme systems in apple tissue and P. digitatum. 2,4-Dinitrofluorobenzene, a **membrane permeant probe**, inhibited ethylene production effectively in apples but far less effectively in P. digitatum. In contrast, salicylaldehyde, another **membrane permeant probe**, effectively inhibited the P. digitatum system but, except at 0.1 mM concentration, little influenced the apple system. 1,5-Difluoro-2,4-dinitrobenzene (DFDNB), a **membrane permeant probe** which cross-links proteins with proteins and with phospholipids, strongly inhibited ethylene biosynthesis in both apple and P. digitatum, whereas dimethyl suberimidate, the protein cross-linking reagent, inhibited slightly the apple system but not P. digitatum system. Picrylsulfonate (TNBS), a non-permeant membrane probe, up to 0.1 mM, did not inhibit any of the 2 systems studied. In the presence of exogenous methionine in the apple system and glutamate in P. digitatum, TNBS at 0.1 and 1 mM caused inhibition of ethylene synthesis. These probes did not affect respiration of apple slices under similar incubating conditions, excepting for DFDNB which on longer incubation did inhibit respiration, but the effect on ethylene synthesis was 15 times greater. Divalent cation ionophores, A23187 and X537 A, had no effect on ethylene synthesis in both the systems. The water soluble iron chelating agent, o-phenanthroline, was a more potent inhibitor of the apple system but minimally affected P. digitatum. In contrast, the lipophilic chelator, bathophenanthroline, was a more potent inhibitor of the P. digitatum system. Assay of the fatty acid composition of polar lipids from crude membrane fractions showed considerably greater linoleic to linolenic ratio in P. digitatum than in apple. It was suggested that the ethylene formation in apple and P. digitatum is sensitive to a modification of membrane structure and that specific chelator-sensitive metals (perhaps Fe and Cu) are involved in ethylene synthesis in both these systems.

L19 ANSWER 9 OF 9 MEDLINE DUPLICATE 7
76237597 Document Number: 76237597. PubMed ID: 947907. Organization of membrane proteins in the intact myelin sheath. Pyridoxal phosphate and salicylaldehyde as probes of myelin structure. Golds E E; Braun P E. JOURNAL OF BIOLOGICAL CHEMISTRY, (1976 Aug 10) 251 (15) 4729-35. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Pyridoxal phosphate and salicylaldehyde were used as protein-labeling probes to study the organization of membrane proteins in the intact myelin sheath of the cat dorsal column. Both reagents react with protein amino groups to form Schiff's bases which can be reduced with NaB3H4. The relatively membrane-impermeant pyridoxal phosphate labels all proteins of the intact myelin except basic protein. This major protein of myelin is labeled only after loss of membrane integrity. The relatively **membrane-permeant probe**, salicylaldehyde, was then used to establish that the basic protein is truly located on the cytoplasmic side of the myelin bilayer, and not merely sequestered within the multiple lamellar structure of the sheath. All proteins in the intact myelin are readily labeled by this reagent, with the label distribution pattern identical to that of disrupted myelin fragments. These data suggest a model for myelin structure in which the basic protein is the only major protein component located exclusively on the cytoplasmic side

of the membrane (the major period zone of the sheath), with the other major proteins disposed wholly, or in part, in the extracellular half of the membrane bilayer (the intraperiod zone). All proteins, although asymmetrically disposed with respect to membrane sidedness, appear to be randomly distributed throughout the lamellae which comprise the sheath.

=> s method of detection
L20 49594 METHOD OF DETECTION

=> s l20 and single chain antibody
L21 32 L20 AND SINGLE CHAIN ANTIBODY

=> s l21 and membrane bound
L22 0 L21 AND MEMBRANE BOUND

=> dup remove l21
PROCESSING COMPLETED FOR L21
L23 32 DUP REMOVE L21 (0 DUPLICATES REMOVED)

=> d l21 1-32 cbib abs

L21 ANSWER 1 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2002:134004 Document No.: PREV200200134004. Recombinant **single chain antibodies** in bioelectrochemical sensors. Benhar, I.; Eshkenazi, I.; Neufeld, T.; Opatowsky, J.; Shaky, S.; Rishpon, J. (1). (1) Department of Molecular Microbiology and Biotechnology, Tel-Aviv University, Ramat Aviv, 69978: rishpon@post.tau.ac.il Israel. Talanta, (13 December, 2001) Vol. 55, No. 5, pp. 899-907. print. ISSN: 0039-9140. Language: English.
AB Recombinant antibodies provide an emerging strategy in the development of new immunosensors. In particular, **single chain antibodies** (scFvs) can be isolated and expressed in bacterial systems that also allow their in vitro manipulation at the gene level. In this work, we present for the first time results of single-chain phage displayed antibodies combined with amperometric detection and its application as an immunosensor. The scFv is immobilized on a carbon electrode and used to capture and quantify its specific target antigen. We describe the detection of the sugar milk lactose, the bacteria Listeria monocytogenes, and the enzyme MtKatG, which is expressed by Mycobacterium tuberculosis.

L21 ANSWER 2 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2001:357629 Document No.: PREV200100357629. Selection and characterisation of recombinant **single-chain antibodies** to the hapten Aflatoxin-B1 from naive recombinant antibody libraries. Moghaddam, Amir (1); Lobersli, Inger; Gebhardt, Kirsti; Braunagel, Michael; Marvik, Ole J.. (1) Affitech AS, Oslo Research Park, Gaustadalleen 21, N-0349, Oslo: amirmo@affitech.com Norway. Journal of Immunological Methods, (1 August, 2001) Vol. 254, No. 1-2, pp. 169-181. print. ISSN: 0022-1759. Language: English. Summary Language: English.
AB Selection of antibodies from large repertoire phage display libraries has become a common technique for isolation of specific antibodies to antigens. Many of these libraries are shown to contain antibodies specific to haptens, but only when these haptens are derivatised or conjugated to an immobilising molecule, such as bovine serum albumin (BSA). There has been little demonstration of the suitability of naive recombinant antibody libraries for isolating antibodies that bind low molecular weight haptens in the absence of a carrier molecule and few have addressed the problems associated with selecting antibodies that only recognize the combination of hapten and the carrier molecule. We have panned two-phage antibody libraries against AflatoxinB1-BSA and screened **single-chain antibody** fragments for binding to AflatoxinB1-BSA

and Aflatoxin-B1. Many of the antibodies isolated specifically bound AflatoxinB1-BSA, but not soluble Aflatoxin-B1 or BSA. Modification of the protocol led to isolation of single-chain fragment variable antibody domain (scFv) antibodies that specifically bound soluble Aflatoxin-B1 with an affinity of 6X10-9 M.

L21 ANSWER 3 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2001:339250 Document No.: PREV200100339250. Genetically fused single-chain anti-Salmonella antibody with aequorin: A bioluminescence immunoassay for a Salmonella antigen. Wang, Jianquan; Ensor, C. Mark; Dubuc, Ginette J.; Narang, Saran A.; Daunert, Sylvia (1). (1) Departments of Chemistry and Pharmaceutical Sciences, University of Kentucky, Lexington, KY, 40506-0055: daunert@pop.uky.edu USA. Analytica Chimica Acta, (24 May, 2001) Vol. 435, No. 2, pp. 255-263. print. ISSN: 0003-2670. Language: English. Summary Language: English.

AB We report the development of a competitive heterogeneous immunoassay for BSA-conjugated Salmonella antigen using a fusion protein between an anti-Salmonella **single-chain antibody** (scFv) and the photo-protein aequorin (AEQ). It has been demonstrated that the fusion protein (scFvLAEQ) maintains both the antibody binding affinity toward the Salmonella antigen as well as the luminescence properties of the photoprotein. Using scFvLAEQ, the competitive immunoassay gave a detection limit of 10 mug ml-1 of Salmonella antigen. The assay eliminates the need for a labeled secondary antibody usually required in enzyme-linked immuosorbent assays (ELISAs), and therefore, it may find potential applications in detecting Salmonella contamination.

L21 ANSWER 4 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2001:322355 Document No.: PREV200100322355. Identification of NB-4 leukemia cell antigens by phage display of antibodies and epitopes. Mullaney, Brian P. (1); Sekella, Phil (1); Green, Gary (1); Pytela, Robert; Pallavicini, Maria G.. (1) Cancer Center, University of California at San Francisco, San Francisco, CA USA. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 826a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology. ISSN: 0006-4971. Language: English. Summary Language: English.

AB Phage display is a powerful combinatorial tool to identify antibody and epitope interactions. For example, an antibody phage display library can be selected against leukemia cells to generate antibodies against intracellular and cell-surface antigens. Characterization of the epitopes recognized by such antibodies may lead to identification of target antigens for diagnostics and/or therapeutics. We used phage display of antibodies and epitopes to identify antigens expressed by NB-4 acute promyelocytic leukemia cells. Rabbits were immunized with undifferentiated and all trans retinoic acid induced differentiated NB-4 cells. An antibody phage display library was constructed from rabbit spleen. Splenic RNA was purified, reverse transcribed, and heavy and light antibody chains amplified by PCR with rabbit specific primers. **Single-chain antibodies** were constructed by overlap extension PCR and cloned into a phagemid vector. Monoclonal antibodies were also produced using novel rabbit hybridoma technologies. Both libraries provide antibodies against novel NB4 cell antigens. Epitope phage display using cDNA can be used to define binding domains of antibody-antigen interactions. In order to demonstrate that phage display is well suited for target identification via antibody - antigen interactions, we generated an epitope display system using an interleukin gene model. A phagemid vector, pORF-1, was designed to display gene fragments. A phage display library was constructed from fragments of the human interleukin-4 cDNA and contained 3 X 10e7 members. This library was selected against C19, an anti-IL-4 antisera, raised against an IL-4 peptide sequence. After two rounds of selection of the epitope library against the antibody, 66/96 individual clones were positive by ELISA. DNA sequencing indentified 5

unique and overlapping clones; corresponding to the C-terminal immunogen epitope. These results validate epitope enrichment by phage display selection against an antibody and form the rationale for construction of NB-4 cDNA epitope libraries for identification of novel antigens. Phage display of antibodies and epitopes may provide a powerful and rapid approach to the study of leukemia proteomics including identification of novel leukemia cell markers.

L21 ANSWER 5 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2001:133745 Document No.: PREV200100133745. GST fusion proteins cause false positives during selection of viral movement protein specific **single chain antibodies**. Zhang, Mei-Yun; Schillberg, Stefan (1); Zimmermann, Sabine; Liao, Yu-Cai; Breuer, Gudrun; Fischer, Rainer. (1) Institut fuer Biologie I (Botanik/Molekulargenetik), RWTH Aachen, Worringerweg 1, 52074, Aachen: schillberg@biol.rwth-aachen.de Germany. Journal of Virological Methods, (February, 2001) Vol. 91, No. 2, pp. 139-147. print. ISSN: 0166-0934. Language: English. Summary Language: English.

AB Glutathione S-transferase (GST) fusion proteins are used frequently for investigating protein-protein and protein-DNA interactions. The present study demonstrates that the use of GST fusion proteins caused false positives during selection of phage-displayed **single-chain antibody** fragments (scFvs) specific for three domains of the movement protein (NSM) of tomato spotted wilt virus (TSWV). To identify and exclude the false positives when using GST as a fusion partner linked to the antigen of interest, indirect phage enzyme-linked immunosorbent assay (ELISA) was compared with capture phage ELISA. Of 210 enriched phage clones, indirect phage ELISA identified 106 clones specific for binding to GST-domain fusions but not to GST. In contrast, using capture phage ELISA, all 106 selected clones were identified as false positives, reacting with the GST fusion proteins and GST. This was confirmed by characterization of soluble scFv antibodies. The data indicate that GST fusion proteins seem unsuitable for screening of phage-displayed antibody fragments and it is essential to use capture phage ELISA, instead of the indirect phage ELISA used commonly to exclude false positives in characterization of selected clones with GST fusion proteins.

L21 ANSWER 6 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2001:86475 Document No.: PREV200100086475. A helper phage to improve **single-chain antibody** presentation in phage display. Rondot, Susanne; Koch, Joachim; Breitling, Frank; Duebel, Stefan (1). (1) Molekulare Genetik, Universitaet Heidelberg, Im Neuenheimer Feld 230, 69120, Heidelberg: sd@uni-hd.de Germany. Nature Biotechnology, (January, 2001) Vol. 19, No. 1, pp. 75-78. print. ISSN: 1087-0156. Language: English. Summary Language: English.

AB We show here that the number of **single-chain antibody** fragments (scFv) presented on filamentous phage particles generated with antibody display phagemids can be increased by more than two orders of magnitude by using a newly developed helper phage (hyperphage). Hyperphage have a wild-type pIII phenotype and are therefore able to infect F+ Escherichia coli cells with high efficiency; however, their lack of a functional pIII gene means that the phagemid-encoded pIII-antibody fusion is the sole source of pIII in phage assembly. This results in an considerable increase in the fraction of phage particles carrying an antibody fragment on their surface. Antigen-binding activity was increased about 400-fold by enforced oligovalent antibody display on every phage particle. When used for packaging a universal human scFv library, hyperphage improved the specific enrichment factor obtained when panning on tetanus toxin. After two panning rounds, more than 50% of the phage were found to bind to the antigen, compared to 3% when conventional M13KO7 helper phage was used. Thus, hyperphage is particularly useful in stoichiometric situations, when there is little chance that a single phage

will locate the desired antigen.

L21 ANSWER 7 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2001:57054 Document No.: PREV200100057054. Isolation of a high affinity scFv from a monoclonal antibody recognising the oncofoetal antigen 5T4. Shaw, D. M. (1); Embleton, M. J.; Westwater, C.; Ryan, M. G.; Myers, K. A.; Kingsman, S. M.; Carroll, M. W.; Stern, P. L.. (1) CRC Immunology Group, Paterson Institute for Cancer Research, Christie Hospital, Manchester, M20 4BX: dshaw@picr.man.ac.uk UK. Biochimica et Biophysica Acta, (15 December, 2000) Vol. 1524, No. 2-3, pp. 238-246. print. ISSN: 0006-3002. Language: English. Summary Language: English.

AB The oncofoetal antigen 5T4 is a 72 kDa glycoprotein expressed at the cell surface. It is defined by a monoclonal antibody, mAb5T4, that recognises a conformational extracellular epitope in the molecule. Overexpression of 5T4 antigen by tumours of several types has been linked with disease progression and poor clinical outcome. Its restricted expression in non-malignant tissue makes 5T4 antigen a suitable target for the development of antibody directed therapies. The use of murine monoclonal antibodies for targeted therapy allows the tumour specific delivery of therapeutic agents. However, their use has several drawbacks, including a strong human anti-mouse immune (HAMA) response and limited tumour penetration due to the size of the molecules. The use of antibody fragments leads to improved targeting, pharmacokinetics and a reduced HAMA. A **single chain antibody** (scFv) comprising the variable regions of the mAb5T4 heavy and light chains has been expressed in Escherichia coli. The addition of a eukaryotic leader sequence allowed production in mammalian cells. The two 5T4 **single chain antibodies**, scFv5T4WT19 and LscFv5T4, described the same pattern of 5T4 antigen expression as mAb5T4 in normal human placenta and by FACS. Construction of a 5T4 extracellular domain-IgGFc fusion protein and its expression in COS-7 cells allowed the relative affinities of the antibodies to be compared by ELISA and measured in real time using a biosensor based assay. MAb5T4 has a high affinity, KD = 1.8 X 10-11 M, as did both **single chain antibodies** , scFv5T4WT19 KD = 2.3 X 10-9 M and LscFv5T4 KD = 7.9 X 10-10 M. The small size of this 5T4 specific scFv should allow construction of fusion proteins with a range of biological response modifiers to be prepared whilst retaining the improved pharmacokinetic properties of scFvs.

L21 ANSWER 8 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2001:52119 Document No.: PREV200100052119. Fully "recombinant enzyme-linked immunosorbent assays" using genetically engineered **single-chain antibody** fusion proteins for detection of Citrus tristeza virus. Terrada, Estela; Kerschbaumer, Randolf J.; Giunta, Giuseppe; Galeffi, Patrizia; Himmler, Gottfried; Cambra, Mariano (1). (1) Instituto Valenciano de Investigaciones Agrarias, Carretera Moncada-Naquera km 4.5, 46113, Moncada, Valencia: mcambra@ivia.es Spain. Phytopathology, (December, 2000) Vol. 90, No. 12, pp. 1337-1344. print. ISSN: 0031-949X. Language: English. Summary Language: English.

AB Recombinant single-chain variable fragment antibodies (scFv) that bind specifically to Citrus tristeza virus (CTV), which cause the most detrimental viral disease in the citrus industry worldwide, were obtained from the hybridoma cell lines 3DF1 and 3CA5. These scFv were genetically fused with dimerization domains as well as with alkaline phosphatase, respectively, and diagnostic reagents were produced by expressing these fusion proteins in bacterial cultures. The engineered antibodies were successfully used for CTV diagnosis in plants by tissue print enzyme-linked immunosorbent assay (ELISA) and double antibody sandwich-ELISA. The fully recombinant ELISAs were as specific and sensitive as conventional ELISAs performed with the parental monoclonal antibodies, showing the usefulness of recombinant antibodies for routine detection of a virus in woody plants for the first time.

L21 ANSWER 9 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2000:482758 Document No.: PREV200000482758. Use of phage display for the generation of human antibodies that neutralize factor IXa function. Suggett, S.; Kirchhofer, D.; Hass, P.; Lipari, T.; Moran, P.; Nagel, M.; Judice, K.; Schroeder, K.; Tom, J.; Lowman, H.; Adams, C.; Eaton, D.; Devaux, B. (1). (1) Genentech, Inc., 1 DNA way, South San Francisco, CA, 94080 USA. Blood Coagulation & Fibrinolysis, (Jan., 2000) Vol. 11, No. 1, pp. 27-42. print. ISSN: 0957-5235. Language: English. Summary Language: English.

AB The use of libraries of phage-displayed human **single-chain antibody** fragments (scFv) has become a new, powerful tool in rapidly obtaining therapeutically useful antibodies. Here, we describe the generation of human scFv and F(ab')2 directed against the gamma-carboxyglutamic acid (Gla) domain of coagulation factor IX. A large library of human scFv, displayed either on M13 phage or expressed as soluble proteins, was screened for binding to human Gla-domain peptide (Tyr1-Lys43). Among a panel of scFv that bound to the factor IX-Gla domain, six scFv clones recognized full-length factor IX and exhibited strong inhibitory activity of factor IX in vitro. After reformatting as F(ab')2, the affinity for factor IX of three selected clones was determined: 10C12 Kd = 1.6 nmol/l, 13D1 Kd = 2.9 nmol/l, and 13H6 Kd = 0.46 nmol/l. The antibodies specifically bound to factor IX and not to other coagulation factors, as assessed by enzyme-linked immunosorbent-type and human plasma clotting assays. The complementarity determining region amino acid sequences of clones 10C12 and 13D1 only differed at a single residue, whereas 13H6 showed little homology, suggesting that 13H6 binds to a different epitope within the factor IX-Gla domain. Despite the slightly lower affinity of 10C12 F(ab')2 versus 13H6 F(ab')2, 10C12 was consistently more potent than 13H6 in prolonging the activated partial thromboplastin time (APTT), in inhibiting platelet-mediated plasma clotting, and in inhibiting factor X activation by the intrinsic Xase complex. Finally, 10C12 F(ab')2 also recognized and neutralized factor IX/factor IXa of different species, as demonstrated by the specific APTT prolongation of dog, mouse, baboon and rabbit plasma. In summary, the results validate the usefulness of scFv phage-displayed libraries to rapidly generate fully human antibodies as potential new therapeutics for thrombotic disorders.

L21 ANSWER 10 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2000:345383 Document No.: PREV200000345383. Divalent forms of CC49 **single-chain antibody** constructs in Pichia pastoris: Expression, purification, and characterization. Goel, Apollina; Beresford, Guy W.; Colcher, David; Pavlinkova, Gabriela; Booth, Barbara J. M.; Baranowska-Kortylewicz, Janina; Batra, Surinder K. (1). (1) Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, 984525 Nebraska Medical Center, Omaha, NB, 68198-4525 USA. Journal of Biochemistry (Tokyo), (May, 2000) Vol. 127, No. 5, pp. 829-836. print. ISSN: 0021-924X. Language: English. Summary Language: English.

AB Single-chain variable fragments (scFvs) are tumor-recognition units that hold enormous potential in antibody-based therapeutics. Their clinical applications, however, require the large scale production and purification of biologically active recombinant scFvs. In the present study, we engineered and expressed divalent non-covalent ((scFv)2-His6) and covalent (sc(Fv)2-His6) scFvs of a tumor-associated monoclonal antibody (MAb) CC49 in Pichia pastoris. The purity and immunoreactivity of the scFvs were analyzed by SDS-PAGE, HPLC, and competitive ELISA. The binding affinity constant (KA), determined by surface plasmon resonance analysis using BIAcore, was 4.28 X 107, 2.75 X 107, and 1.14 X 108 M-1 for (scFv)2-His6, sc(Fv)2-His6, and CC49 IgG, respectively. The expression of scFvs in P. pastoris was 30 to 40-fold higher than in Escherichia coli. Biodistribution studies in athymic mice bearing LS-174T human colon carcinoma xenografts showed equivalent tumor-targeting of CC49 dimers generated in yeast (scFv)2-His6 and bacteria (scFv)2 with 12.52% injected

dose/gram (%ID/g) and 11.42%ID/g, respectively, at 6 h post-injection. Interestingly, the pharmacokinetic pattern of dimeric scFvs in xenografted mice exhibited a slower clearance of His-tagged scFvs from the blood pool than scFvs lacking the His-tag (0.1 gtoreq p gtoreq 0.05). In conclusion, improved yields of divalent scFvs were achieved using the P. pastoris expression/secretion system. The in vitro and in vivo properties of these scFvs suggest possible therapeutic applications.

L21 ANSWER 11 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2000:260411 Document No.: PREV200000260411. In vivo selection of **single-chain antibodies** using a yeast two-hybrid system. Portner-Taliana, Antje (1); Russell, Marijane; Froning, Karen J.; Budworth, Paul R.; Comiskey, John D.; Hoeffler, James P.. (1) Invitrogen, 1600 Faraday Avenue, Carlsbad, CA, 92008 USA. Journal of Immunological Methods, (April 21, 2000) Vol. 238, No. 1-2, pp. 161-172. print.. ISSN: 0022-1759. Language: English. Summary Language: English.
AB The current methodology for screening libraries of single-chain fragments of immunoglobulin variable domains (sFvs) utilizes bacterial phage systems. We have developed a unique in vivo selection protocol combining a modified yeast two-hybrid assay with a novel prey vector expressing sFvs. The viability of the system is demonstrated with the screen of a sFv library cloned into a yeast two-hybrid prey vector for molecules that target the bait ATF-2, a member of the CREB/ATF family of transcriptional regulatory proteins. The isolated sFv was capable of recognizing ATF-2 in vitro on Western blots and in vivo in mammalian cells.

L21 ANSWER 12 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2000:233582 Document No.: PREV200000233582. Targeting retrovirus to cancer cells expressing a mutant EGF receptor by insertion of a **single chain antibody** variable domain in the envelope glycoprotein receptor binding lobe. Lorimer, Ian A. J. (1); Lavictoire, Sylvie J.. (1) Cancer Research Group, Ottawa Regional Cancer Centre, 501 Smyth Road, Ottawa, ON, K1H 8L6 Canada. Journal of Immunological Methods, (April 3, 2000) Vol. 237, No. 1-2, pp. 147-157. ISSN: 0022-1759. Language: English. Summary Language: English.
AB We have investigated targeting of retroviral vectors to a mutant EGF receptor (EGFRvIII) that is expressed in cancers of the brain, breast, lung and ovary, but is not found in any normal tissues. An expression plasmid was made in which a single chain Fv antibody specific for EGFRvIII was inserted at a novel position within a disulphide-bonded surface loop near the native receptor binding site of the Moloney leukemia virus ecotropic envelope glycoprotein. This fusion protein was expressed and incorporated into retroviral particles as efficiently as normal envelope glycoprotein. Retroviral vectors made with the fusion protein were able to bind peptide antigen and EGFRvIII expressed on the surface of human glioblastoma cells. The retroviral vectors had normal levels of infectivity on mouse cells, showing that the envelope glycoprotein tolerated a large insertion at this site, but did not show significant infectivity to human cells expressing EGFRvIII. Thus we were able to redirect retrovirus binding to this tumour-specific target without perturbing the normal function of the ecotropic envelope glycoprotein, but this was not sufficient to mediate infectivity via this receptor.

L21 ANSWER 13 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2000:196445 Document No.: PREV200000196445. Development and characterization of a bispecific **single-chain antibody** directed against T cells and ovarian carcinoma. Kriangkum, Jitra; Xu, Biwen; Gervais, Christian; Paquette, Denis; Jacobs, Frederik A.; Martin, Luis; Suresh, Mavanur R. (1). (1) Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, T6G2N8 Canada. Hybridoma, (Feb., 2000) Vol. 19, No. 1, pp. 33-41. ISSN: 0272-457X. Language: English. Summary Language: English.
AB Bispecific antibodies with specificity for tumor antigen and CD3 have been

shown to redirect the cytotoxicity of T cells against relevant tumor. Our objective was to generate single-chain bispecific antibodies (bsSCA) that could retarget mouse cytotoxic T lymphocytes (CTL) to destroy human ovarian carcinoma in a xenogeneic setting. A bsSCA, 2C11 X B43.13, was constructed by genetic engineering and expressed in mammalian cells. Molecular characteristics, binding properties, and ability to retarget CTL were studied. Western blot analysis showed that the product is a 65-kDa protein. Purification of antibodies could be done by single-step affinity chromatography using protein L-agarose with an unoptimized yield of 200 mug/L. BsSCA 2C11 X B43.13 was capable of binding to mouse CD3 and human CA125 as detected by FACS analysis of EL4 and OVCAR Nu3H2 cells, respectively. It could also bridge activated splenic T cells and human ovarian carcinoma as demonstrated by a bridge FACS assay. Redirected mouse CTL could mediate human target cell lysis in a 20-h 51Cr release assay despite that they are xenogeneic. Prolonged incubation of redirected CTL and tumor targets resulted in a dramatic reduction in tumor cell number. CD28 co-stimulation enhanced redirected CTL function in both types of assays. BsCA 2C11 X B43.13 thus can be used as a preclinical immunotherapeutic model for human ovarian cancer in a xenogeneic setting.

L21 ANSWER 14 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2000:114058 Document No.: PREV200000114058. Production and in vitro refolding of a **single-chain antibody** specific for human plasma apolipoprotein A-I. Cho, Won-Kyung; Sohn, Uik; Kwak, Ju-Won (1). (1) Cardiovascular Research Laboratory, Korea Research Institute of Bioscience and Biotechnology, Yuseong, Taejon, 305-600 South Korea. Journal of Biotechnology, (Feb. 17, 2000) Vol. 77, No. 2-3, pp. 169-178. ISSN: 0168-1656. Language: English. Summary Language: English.
AB An active form of **single-chain antibody** (scFv) has been produced in Escherichia coli for murine monoclonal antibody MabA34 (gamma1, kappa), which is specific for human plasma apolipoprotein (apo) A-I. The complementary DNAs (cDNAs) encoding the variable regions of heavy chain (VH) and light chain (VL) were connected by a (Gly4Ser)3 linker using an assembly polymerase chain reaction. The construct (VL-linker-VH) was placed under the control of highly efficient T7 promoter system. The cloned scFv was expressed in E. coli as inclusion bodies. After purification from E. coli lysate using sonication and low speed centrifugation, the inclusion body was solubilized and denatured in the presence of 8 M urea, renatured by dialysis, and scFv was finally purified using antigen-affinity chromatography. The purity and activity of purified scFv were confirmed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), Western blotting and enzyme-linked immunosorbent assay (ELISA). The affinity constant was determined by a biosensor method using the BIAcore system. The results showed that the yield of correctly refolded scFv was more than 20 mg l-1 of E. coli flask culture and the specific binding activity to apo A-I was retained with an affinity constant of 6.74 X 10-8 M (Kd). A notable thing is that guanidine-HCl as a denaturant induced more multimeric formation in the subsequent refolding procedure for the scFv of MabA34 and thus, it was not suitable as urea was. This fact is uncommon for what is generally known for the denaturation and refolding of recombinant antibodies.

L21 ANSWER 15 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2000:91721 Document No.: PREV200000091721. Inhibition of expression of the Galalpha1-3Gal epitope on porcine cells using an intracellular **single-chain antibody** directed against alpha1,3Galactosyltransferase. Sepp, Armin; Farrar, Conrad A.; Dorling, Tony; Cairns, Tom; George, Andrew J. T.; Lechler, Robert I. (1). (1) Department of Immunology, Division of Medicine, Imperial College School of Medicine, Du Cane Road, Hammersmith Campus, London, W12 0NN UK. Journal of Immunological Methods, (Dec. 10, 1999) Vol. 231, No. 1-2, pp. 191-205. ISSN: 0022-1759. Language: English. Summary Language: English.
AB The carbohydrate epitope Galalpha-1-3Gal has been shown to be the major

target of natural antibodies responsible for hyperacute rejection of porcine tissues transplanted into primates. We have sought to produce a phenotypic knockout of the alpha1,3Galactosyltransferase enzyme that is responsible for generating this epitope, using an intracellular antibody approach. We have isolated high affinity anti-alpha1,3Galactosyltransferase **single-chain antibodies** from a semi-synthetic phage display library. Expression of a KDEL-tagged anti-alpha1,3Galactosyltransferase **single-chain antibody** in a porcine endothelial cell line resulted in the decreased expression of the Galalpha1-3Gal epitope and increased resistance to lysis by human serum.

L21 ANSWER 16 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2000:37623 Document No.: PREV200000037623. Matrix-assisted refolding of single-chain Fv-cellulose binding domain fusion proteins. Berdichevsky, Yevgeny; Lamed, Raphael; Frenkel, Dan; Gophna, Uri; Bayer, Edward A.; Yaron, Sima; Shoham, Yuval; Benhar, Itai (1). (1) Tel-Aviv University, Green Bldg., Room 202, Ramat Aviv, 69978 Israel. Protein Expression and Purification, (Nov., 1999) Vol. 17, No. 2, pp. 249-259. ISSN: 1046-5928. Language: English. Summary Language: English.

AB We describe a method for the isolation of recombinant **single-chain antibodies** in a biologically active form. The **single-chain antibodies** are fused to a cellulose binding domain as a single-chain protein that accumulates as insoluble inclusion bodies upon expression in Escherichia coli. The inclusion bodies are then solubilized and denatured by an appropriate chaotropic solvent, then reversibly immobilized onto a cellulose matrix via specific interaction of the matrix with the cellulose binding domain (CBD) moiety. The efficient immobilization that minimizes the contact between folding protein molecules, thus preventing their aggregation, is facilitated by the robustness of the Clostridium thermocellum CBD we use. This CBD is unique in retaining its specific cellulose binding capability when solubilized in up to 6 M urea, while the proteins fused to it are fully denatured. Refolding of the fusion proteins is induced by reducing with time the concentration of the denaturing solvent while in contact with the cellulose matrix. The refolded **single-chain antibodies** in their native state are then recovered by releasing them from the cellulose matrix in high yield of 60% or better, which is threefold or higher than the yield obtained by using published refolding protocols to recover the same scFvs. The described method should have general applicability for the production of many protein-CBD fusions in which the fusion partner is insoluble upon expression.

L21 ANSWER 17 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2000:27326 Document No.: PREV200000027326. Apoplastic and cytosolic expression of full-size antibodies and antibody fragments in Nicotiana tabacum. Schillberg, Stefan (1); Zimmermann, Sabine; Voss, Andreas; Fischer, Rainer. (1) Fraunhofer Abteilung fuer Molekulare Biotechnologie, IUCT, Grafschaft, Auf dem Aberg 1, D-57392, Schmallenberg Germany. Transgenic Research, (Aug., 1999) Vol. 8, No. 4, pp. 255-263. ISSN: 0962-8819. Language: English. Summary Language: English.

AB We compared the expression of a functional recombinant TMV-specific full-size antibody (rAb29) in both the apoplast and cytosol of tobacco plants and a **single chain antibody** fragment (scFv29), derived from rAb29, was expressed in the cytosol. Cloned heavy and light chain cDNAs of full-size rAb29, which binds to TMV coat protein monomers, were integrated into the plant expression vector pSS. The full-size rAb29 was expressed in the cytosol and targeted to the apoplast by including the original murine antibody leader sequences. Levels of functional full-size rAb29 expression were high in the apoplast (up to 8.5 mug per gram leaf tissue), whereas cytosolic expression was low or at the ELISA detection limit. Sequences of the variable domains of rAb29 light and heavy chain were used to generate the **single chain**

antibody scFv29, which was expressed in the periplasmic space of E. coli and showed the same binding specificity as full-size rAb29. In addition, scFv29 was functionally expressedin the cytosol of tobacco plants and plant derived scFv29 maintained same binding specificity to TMV-coat protein monomers as rAb29.

L21 ANSWER 18 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1999:526283 Document No.: PREV199900526283. In vitro folding and thermodynamic stability of an antibody fragment selected in vivo for high expression levels in Escherichia coli cytoplasm. Martineau, Pierre (1); Betton, Jean-Michel. (1) Faculte de Pharmacie, CNRS UMR9921, 15 avenue Charles Flahault, 34060, Montpellier Cedex 2 France. Journal of Molecular Biology, (Oct. 1, 1999) Vol. 292, No. 4, pp. 921-929. ISSN: 0022-2836. Language: English. Summary Language: English.

AB We recently isolated a mutant of a human anti-beta-galactosidase **single chain antibody** fragment (scFv) able to fold at high levels in Escherichia coli cytoplasm. When targeted to the periplasm, this mutant and the wild-type scFv are both expressed at comparable levels in a soluble, active and oxidized form. If a reducing agent is added to the growth medium, only the mutant scFv is still able to fold, showing that in vivo aggregation is a direct consequence of the lack of disulphide bond formation and not of the cellular localization. In vitro denaturation/renaturation experiments show that the mutant protein is more stable than the wild-type scFv. Furthermore, refolding kinetics under reducing conditions show that the mutant folds faster than the wild-type protein. Aggregation does not proceed from the native or unfolded conformation of the protein, but from a species only present during the unfolding/refolding transition. In conclusion, the in vivo properties of the mutant scFv can beexplained by, first, an increase in the stability of the protein in order to tolerate the removal of the two disulphide bonds and, second, a modification of its folding properties that reduces the kinetic competition between folding and aggregation of a reduced folding intermediate.

L21 ANSWER 19 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1999:402594 Document No.: PREV199900402594. Single-chain Fv fragment of catalytic antibody 4f4f with glycosidase activity: Design, expression, and purification. Jang, Chang Hwan; Chung, Hyun-Ho; Yu, Jaehoon; Chang, Yung-Jin; Kim, Hyong Bai; Paek, Se-Hwan; Shin, Dong-Hoon; Kim, Kyung Hyun (1). (1) Graduate School of Biotechnology, Korea University, Seoul, 136-701 South Korea. Journal of Microbiology and Biotechnology, (June, 1999) Vol. 9, No. 3, pp. 376-380. ISSN: 1017-7825. Language: English. Summary Language: English.

AB Constructs, encoding a single-chain variable fragment of a catalytic antibody 4f4f (scFv-4f4f) with glycosidase activity, were made by combining the coding sequences for the heavy and light chain variable domains with a sequence encoding a linker (GGGGS). Using three different plasmid systems, **single-chain antibodies** were expressed separately in Escherichia coli, demonstrating significant differences in the expression level and amounts in soluble form of the recombinant protein. The protein expression from pET3a-scFv-4f4f was up to 20% of the total soluble proteins and, more importantly, the proteins were mostly found in a soluble form. An SDS-PAGE analysis of the purified single-chain proteins, yielding higher than 5 mg from a 1-1 culture, showed a single band corresponding to its molecular weight of 29,100. A preliminary study shows that the expressed scFv-4f4f is catalytically active. The catalytic parameters for the hydrolysis of p-nitrophenyl-beta-D-glucopyranoside by scFv-4f4f are being investigated.

L21 ANSWER 20 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1999:281464 Document No.: PREV199900281464. A recombinant, fully human monoclonal antibody with antitumor activity constructed from phage-displayed antibody fragments. Huls, Gerwin A.; Heijnen, Ingmar A. F.

M.; Cuomo, Maria E.; Koningsberger, Jacob C.; Wiegman, Luus; Boel, Edwin; van der Vuurst de Vries, Anne-Renee; Loyson, Sabine A. J.; Helfrich, Wijnand; van Berge Henegouwen, Gerard P.; van Meijer, Marja; de Kruif, John; Logtenberg, Ton (1). (1) Department of Immunology, University Hospital Utrecht, Utrecht Netherlands. Nature Biotechnology, (March, 1999) Vol. 17, No. 3, pp. 276-281. ISSN: 1087-0156. Language: English. Summary Language: English.

AB A single-chain Fv antibody fragment specific for the tumor-associated Ep-CAM molecule was isolated from a semisynthetic phage display library and converted into an intact, fully human IgG1 monoclonal antibody (huMab). The purified huMab had an affinity of 5 nM and effectively mediated tumor cell killing in in vitro and in vivo assays. These experiments show that nonimmunized phage antibody display libraries can be used to obtain high-affinity, functional, and clinically applicable huMabs directed against a tumor-associated antigen.

L21 ANSWER 21 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

1999:273904 Document No.: PREV199900273904. Site-specific modification of a **single-chain antibody** using a novel glyoxylyl-based labeling reagent. Zhao, Zhan G.; Im, Jin S.; Lam, Kit S. (1); Lake, Douglas F. (1). (1) Arizona Cancer Center, 1515 North Campbell, Tucson, AZ, 85724 USA. Bioconjugate Chemistry, (May-June, 1999) Vol. 10, No. 3, pp. 424-430. ISSN: 1043-1802. Language: English. Summary Language: English.

AB A novel, highly specific protein modification approach is described. By using conventional molecular cloning techniques, a protein can be constructed and expressed such that the N-terminal residue is replaced by cysteine. Its 1,2-aminothiol structure reacts very specifically with a glyoxylyl group at pH 7 or below, forming a relatively stable thiazolidine bridge. Therefore, a glyoxylyl-based labeling agent (e.g., radioactive tags, fluorescent probes, biotin) can be used to specifically modify a protein at its N-terminus. To highlight this novel approach, a recombinant anti-insulin **single chain antibody** (scFv) was specifically biotinylated at its N-terminus even in the presence of other proteins in the total cell lysate. The glyoxylyl-biotinylated scFv retained binding activity similar to unmodified scFv.

L21 ANSWER 22 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

1999:241483 Document No.: PREV199900241483. Escherichia coli Skp chaperone coexpression improves solubility and phage display of **single-chain antibody** fragments. Hayhurst, Andrew; Harris, William J. (1). (1) Department of Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD UK. Protein Expression and Purification, (April, 1999) Vol. 15, No. 3, pp. 336-343. ISSN: 1046-5928. Language: English. Summary Language: English.

AB Expression of **single-chain antibody** fragments (scAb) in the periplasm of Escherichia coli often results in low soluble product yield and cell lysis. We have increased scAb solubility and prevented cell culture lysis by coexpressing the E. coli Skp chaperone gene. A mutant Skp cistron was linked to a bacteriophage T7 gene 10 translational initiation region and placed either downstream of a scAb gene within an isopropyl beta-D-thiogalactopyranoside-inducible expression cassette or on a separate colE1-compatible arabinose-inducible vector. Increases in scAb solubility reflected the amount of coexpressed Skp. A bacteriophage display vector that was also engineered to coexpress Skp permitted display of a virtually undisplayable scAb and should prove useful in expanding library sizes.

L21 ANSWER 23 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

1999:227325 Document No.: PREV199900227325. Selection of an anti-CD20, **single-chain antibody** by phage ELISA on fixed cells. Schmidt, Stefanie; Braunagel, Michael; Kuerschner, Timo; Little, Melvyn (1). (1) German Cancer Research Center, FSP4/D0500, Im Neuenheimer

Feld 280, 69120, Heidelberg Germany. Biotechniques, (April, 1999) Vol. 26, No. 4, pp. 697-702. ISSN: 0736-6205. Language: English. Summary Language: English.

AB Cloning the correct genes that code for antibody-variable domains from hybridomas is often complicated by the presence of several immunoglobulin transcripts, some of them arising from a myeloma cell line. For the rapid functional evaluation of recombinant antibody fragments against cell-surface antigens, we established an efficient expression and screening system using phagemid antibodies and fixed cells. VL and VH-polymerase chain reaction (PCR) products, amplified from hybridoma cDNA, were cloned into the phagemid vector pSEX81. After transduction into E. coli and phage rescue, clones were tested for antigen binding using a phage-enzyme-linked immunosorbent assay (ELISA) procedure with whole cells fixed to ELISA wells. This procedure facilitated the successful cloning of a functional anti-CD20, **single-chain antibody** from hybridoma cDNA. The CD20 B-lymphocyte surface antigen expressed by B-cell lymphomas is an attractive target for cancer treatment using immunoconjugates or bi-specific antibodies.

L21 ANSWER 24 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

1999:136209 Document No.: PREV199900136209. Intrathymic function of the human cortical epithelial cell surface antigen gp200-MR6: **Single-chain antibodies** to evolutionarily conserved determinants disrupt mouse thymus development. Palmer, D. B. (1); Crompton, T.; Marandi, M. B.; George, A. J. T.; Ritter, M. A.. (1) Dep. Immunology, Div. Med., Imperial Coll. Sch. Med., Hammersmith Hosp., Du Cane Road, London W12 0NN UK. Immunology, (Feb., 1999) Vol. 96, No. 2, pp. 236-245. ISSN: 0019-2805. Language: English.

AB The mouse monoclonal antibody MR6 recognizes a 200000 MW protein (gp200-MR6), which is expressed highly on human thymic cortical epithelial cells. The antigen is also expressed on some epithelial turnouts and we have previously shown that MR6 inhibits the proliferation of the colon carcinoma cell lines HT29. However, the role of this molecule in the thymus is not known. In order to generate reagents that could be used in murine thymic functional studies we isolated antibodies specific to human gp200-MR6, using a phage display library expressing single-chain (sFv) antibodies. Three independent clones were isolated by panning with purified protein and their specificity was confirmed by immunohistochemistry, Western blotting and flow cytometry. In addition to human thymus, these phage antibodies also recognized the homologous antigen in mouse, pig and other species. Expressed as soluble sFv one of these clones inhibited the proliferation of HT29 cells and a mouse thymic epithelial cell line, suggesting that this antibody exhibits similar functional activity to MR6. In fetal thymic organ culture, thymocytes recovered from thymic lobes cultured in the presence of this sFv, were reduced in number fivefold compared with the control and the majority remained at the double-negative stage of development. These data indicate that gp200-MR6 plays an important role in thymocyte development. In addition, this is the first report to demonstrate that specific sFv can be used to study, and alter, thymic development. This work also highlights the advantage of phage antibody technology in selecting such reagents for functional assays.

L21 ANSWER 25 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

1999:78852 Document No.: PREV199900078852. Selection of human anti-human immunodeficiency virus type 1 envelope **single-chain antibodies** from a peripheral blood cell-based phage repertoire. de Haard, J. J. W. (1); Kazemier, B.; Oudshoorn, P.; Boender, P.; Van Gemen, B.; Koolen, M. J. M.; Van Der Groen, G.; Hoogenboom, H. R.; Arends, J. W.. (1) Dep. Functional Biomolecules, Unilever Res. Lab. Vlaardingen, PO Box 114, 3130 AC Vlaardingen Netherlands. Journal of General Virology, (Dec., 1998) Vol. 79, No. 12, pp. 2883-2894. ISSN: 0022-1317. Language: English.

AB Monoclonal antibodies play an important role in the development of

diagnostic assays. Instead of using hybridoma technology to isolate human immunodeficiency virus type 1-specific antibodies, a phage-displayed antibody library was generated from a small number (107) of peripheral blood lymphocytes from a seropositive donor. Two families of **single-chain antibodies** (scFvs) were selected by biopanning with the envelope precursor gp160. ELISA and competition in the BIAcore system revealed that one antibody family recognized a conformation-sensitive epitope within gp120, while the other antibody family was gp41-specific. The latter group had sequence similarity to antibodies recognizing the cluster III epitope of gp41. Binding of scFvs to gp160 could be inhibited with the donor's serum antibodies, indicating that antibodies with a similar specificity were circulating in the donor's blood. Competition experiments suggested that the epitope of the anti-gp41 antibodies was recognized by a broad range of patients' sera: 21 out of 22 sera from North American and all 20 sera from African seropositive patients inhibited binding of scFvs. In contrast, three sera from this panel did not react with the epitope of the anti-gp120 antibodies. These data indicate that, because of the conserved nature of its epitope, the anti-gp41 antibody will be suitable for diagnostic applications.

L21 ANSWER 26 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1999:72996 Document No.: PREV199900072996. Antiferritin **single-chain antibody**: A functional protein with incomplete folding. Martsev, Sergey P. (1); Kravchuk, Zinaida I.; Chumanevich, Alexander A.; Vlasov, Alexander P.; Dubnovitsky, Anatoly P.; Bespalov, Ivan A.; Arosio, Paolo; Deyev, Sergey M.. (1) Inst. Bio-Organic Chem., Natl. Acad. Sci. Belarus, Zhodinskaya 5/2, Minsk 220141 Belarus. FEBS Letters, (Dec. 28, 1998) Vol. 441, No. 3, pp. 458-462. ISSN: 0014-5793. Language: English.

AB The pET(scF11) plasmid was constructed comprising the gene of a **single-chain antibody** against human ferritin. This plasmid encodes the leader peptide pelB followed by the heavy chain variable VH domain, (Gly4Ser)3 linker peptide, and light chain variable VL domain. The correctly processed scF11 antibody was expressed in Escherichia coli as an insoluble protein without the leader peptide. Purified soluble seF11 was obtained after solubilization in 6 M GdnHCl followed by a sequential dialysis against decreasing urea concentrations and ion-exchange chromatography. ScF11 demonstrated only a apprx 8-fold decrease in the affinity (Ka = 5.1 X 108 M-1 in RIA and 1.8 X 108 M-1 in ELISA) vs. the parent IgG2a/kappa monoclonal antibody F11. The emission maximum of intrinsic fluorescence strongly suggests a compact conformation with tryptophanyl fluorophores buried in the protein interior, consistent with the functionality of the protein. However, scF11 demonstrated (i) the lack of denaturant-induced fluorescence 'dequenching' effect characteristic of the completely folded parent antibody, and (ii) prominent binding, under physiological conditions, of a hydrophobic probe 8-anilino-1-naphthalenesulfonate (ANS) recognizing partially structured states of a protein. These findings are indicative of an incomplete tertiary fold that gives ANS access to the protein hydrophobic core. This work provides the first indication that the functional **single-chain antibody** scF11 displays some properties of a partially structured state and therefore may possess incomplete folding.

L21 ANSWER 27 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1999:63680 Document No.: PREV199900063680. Modulation of antibody display on M13 filamentous phage. Chappel, J. A.; He, M.; Kang, A. S. (1). (1) Dep. Molecular Biology, Scripps Res. Inst., 10550 North Torrey Pines Road, La Jolla, CA 92037 USA. Journal of Immunological Methods, (Dec., 1998) Vol. 221, No. 1-2, pp. 25-34. ISSN: 0022-1759. Language: English.

AB Here we describe a phage vector for the display of **single chain antibodies** and polypeptides on the surface of filamentous M13 phage which permits facile manipulation of the valency of display. The gene encoding the polypeptide is fused to a synthetic copy of

the major coat protein VIII gene (gpVIII) which permits incorporation into the phage during assembly of the filament. Here we examine the growth parameters of phage propagation on the subsequent selection of an anti-progesterone antibody fragment from a mixture of display phage. Our results suggest that the density of the polypeptides displayed on phage may be modulated by altering growth conditions. This ability to influence polypeptide display density on filamentous phage may provide a versatile approach for accessing complex libraries and the capture of weaker ligand receptor interactions by avidity, whilst the potential to access and discriminate between higher affinity interactions is not negated.

L21 ANSWER 28 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1998:473101 Document No.: PREV199800473101. Thermodynamics and kinetics of the reaction of a **single-chain antibody** fragment (scFv) with the leucine zipper domain of transcription factor GCN4. Weber-Bornhauser, Susanne; Eggenberger, Jolanda; Jelesarov, Ilian; Bernard, Andre; Berger, Christine; Bosshard, Hans Rudolf (1). (1) Biochem. Inst., Univ. Zurich, Winterthurerstr. 190, CH-8057 Zurich Switzerland. Biochemistry, (Sept. 15, 1998) Vol. 37, No. 37, pp. 13011-13020. ISSN: 0006-2960. Language: English.

AB Single-chain Fv (scFv) fragments of antibodies have become important analytical and therapeutic tools in biology and medicine. The reaction of scFv fragments has not been well-characterized with respect to the energetics and kinetics of antigen binding. This paper describes the thermodynamic and kinetic behavior of the high-affinity scFv fragment SW1 directed against the dimeric leucine zipper domain of the yeast transcription factor GCN4. The scFv fragment was selected by the phage display technique from the immune repertoire of a mouse that had been immunized with the leucine zipper domain of GCN4. The scFv fragment was produced in high yield in Escherichia coli inclusion bodies and refolded from the denatured state. Differential scanning calorimetry showed that SWI was stable up to about 50 degreeC, but the subsequent thermal denaturation was irreversible (Tm approximately 68degreeC). The scFv fragment specifically recognized the dimeric leucine zipper conformation. Two scFv fragments bound to the GCN4 dimer to form the complex (scFv)2-GCN4. Because of its repetitive structure, the rod-shaped GCN4 leucine zipper may present two similar epitopes for the scFv fragment. Surprisingly, the binding reaction was highly cooperative, that is, the species (scFv)2-GCN4 dominated over scFv-GCN4 even in the presence of a large excess of the antigen GCN4. It is speculated that cooperativity resulted from direct interaction between the two GCN4-bound scFv fragments. At 25degreeC, the average binding enthalpy for a scFv fragment was favorable (-61 kJ mol-1), the entropy change was unfavorable, and the change in heat capacity was -1.27 +- 0.14 kJ mol-1 K-1. As a result of enthalpy-entropy compensation, the free binding energy was virtually independent of temperature in the physiological temperature range. Antigen binding in solution could be described by a single-exponential reaction with an apparent rate constant of 1 X 106 M-1 s-1. Binding followed in a biosensor with the dimeric GCN4 coupled to the surface of the metal oxide sensor chip was 20 times slower.

L21 ANSWER 29 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1998:472615 Document No.: PREV199800472615. Increasing the secretory capacity of Saccharomyces cerevisiae for production of **single-chain antibody** fragments. Shusta, Eric V.; Rianes, Ronald T.; Plueckthun, Andreas; Wittrup, K. Dane (1). (1) Dep. Chemical Engineering, Univ. Ill., Urbana, IL 61801 USA. Nature Biotechnology, (Aug., 1998) Vol. 16, No. 8, pp. 773-777. ISSN: 1087-0156. Language: English.

AB We have produced **single-chain antibody** fragments (scFv) in Saccharomyces cerevisiae at levels up to 20 mg/L in shake flask culture by a combination of expression level tuning and overexpression of folding assistants. Overexpression of the chaperone BiP

or protein disulfide isomerase (PDI) increases secretion titers 2-8 fold for five scFvs. The increases occur for scFv expression levels ranging from low copy to ER-saturating overexpression. The disulfide isomerase activity of PDI, rather than its chaperone activity, is responsible for the secretion increases. A synergistic increase in scFv production occurs upon coover-expression of BiP and PDI.

L21 ANSWER 30 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1998:457284 Document No.: PREV199800457284. Purification, characterization, and biotinylation of **single-chain antibodies**
. Kipriyanov, Sergey M. (1). (1) Recombinant Antibody Res. Group, Ger. Cancer Res. Cent., Heidelberg Germany. Reischl, U. [Editor]. Methods in Molecular Medicine, (1998) Vol. 13, pp. 615-622. Methods in Molecular Medicine; Molecular diagnosis of infectious diseases. Publisher: Humana Press Inc. Suite 808, 999 Riverview Drive, Totowa, New Jersey 07512, USA. ISBN: 0-89603-398-8 (paper), 0-89603-485-2 (cloth). Language: English.

L21 ANSWER 31 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1998:341462 Document No.: PREV199800341462. Multifunctional g3p-peptide tag for current phage display systems. Beckmann, Christiane; Haase, Bernd; Timmis, Kenneth N.; Tesar, Michael (1). (1) Div. Microbiol., GBF-Natl. Res. Centre Biotechnol., Mascheroder Weg 1, D-38124 Braunschweig Germany. Journal of Immunological Methods, (March 15, 1998) Vol. 212, No. 2, pp. 131-138. ISSN: 0022-1759. Language: English.

AB We have previously described a monoclonal antibody (mAb), 10C3, directed against the gene-3 protein (g3p) of filamentous phage M13, which was produced to study g3p fusion protein expression in Escherichia coli and its incorporation in the phage capsid (Tesar, M., Beckmann, C., Rottgen, P., Haase, B., Faude, U., Timmis, K., 1995. Monoclonal antibody against pIII of filamentous phage: an immunological tool to study pIII fusion protein expression in phage display systems. Immunology 1, 53-54). In this study we report mapping of the antigenic epitope of the mAb 10C3, by means of short overlapping peptide-sequences (Frank, R., Overwin, H., 1996. Spot synthesis. In: Morris, G.E. (Ed.), Methods in Molecular Biology, Vol. 66: Epitope Mapping Protocols. Humana Press, Totowa, NJ, pp. 149-169.) comprising the C-terminal half of the g3-protein. A minimal recognizable peptide was found which is represented in the 11 amino acid sequence from positions 292 to 302 of g3p (Wezenbeek van, P.M.G.P., Hulsebos, T.J.M., Schoenmakers, J.G.G., 1980. Nucleotide sequence of the filamentous bacteriophage Ml 3 DNA genome: comparison with phage fd. Gene II, 129-148). In order to use the antibody also for detection and purification of recombinant proteins, such as **single chain antibodies**, the epitope was introduced as a tag sequence into the phagemid pHEN) (Hoogenboom, H.R., Griffith, A.D., Johnson, K., Chiswell, D.J., Hudson, P., Winter, G., 1991. Multi-subunit proteins on the surface of the filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains. Nucleic Acid Res. 19, 4133-4137; Nissim, A., Hoogenboom, H.R., Tomlinson, I.M., Flynn, G., Midgley, C., Lane, D., Winter. G., 1994. Antibody fragments from a single pot phage display library as immunochemical reagents. EMBO J. 13 (3) 692-698). Purified **single chain antibodies** containing this tag were detectable down to a concentration of 2 mg ml-1 under non-denaturing conditions (ELISA) or 4 ng per lane on immunoblots. The high sensitivity of the antibody for the peptide tag was reflected in the antibody affinity constant KD of 6.80 X 10-10 M, which was determined by real time biomolecular interaction analysis (BIA) based on surface plasmon resonance (SPR) (Karlsson, R., Falt, A., 1997. Experimental design for kinetic analysis of protein-protein interactions with surface plasmon resonance biosensors. J. Immunol. Methods 200, 121-133). Finally, recombinant proteins in E. coli periplasmic extracts could be purified in a single step-by affinity purification using immobilized mAb 10C3. These studies demonstrated that the new peptide-tag and its corresponding mAb represents a versatile tool for the detection of recombinant proteins selected by

phage display technology.

L21 ANSWER 32 OF 32 SCISEARCH COPYRIGHT 2003 ISI (R)
2002:810412 The Genuine Article (R) Number: 599LL. HER-2 in breast cancer - **methods** of **detection**, clinical significance and future prospects for treatment. Rampaul R S; Pinder S E; Gullick W J; Robertson J F R; Ellis I O (Reprint). City Hosp Nottingham, Dept Pathol, Hucknall Rd, Nottingham NG1 5PB, England (Reprint); City Hosp Nottingham, Dept Pathol, Nottingham NG1 5PB, England; City Hosp Nottingham, Dept Surg, Nottingham NG5 1PB, England; Univ Kent, Sch Biol Sci, Canterbury CT2 7NZ, Kent, England. CRITICAL REVIEWS IN ONCOLOGY HEMATOLOGY (SEP 2002) Vol. 43, No. 3, pp. 231-244. Publisher: ELSEVIER SCIENCE INC. 360 PARK AVE SOUTH, NEW YORK, NY 10010-1710 USA. ISSN: 1040-8428. Pub. country: England. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The Human Epidermal Growth Factor (HER-2) oncogene encodes a transmembrane tyrosine kinase receptor with extensive homology to the Epidermal Growth Factor Receptor (EGFR) which is the prototypal member of this family of receptor tyrosine kinases. HER-2 gene amplification is found in 20-30% of breast cancers. Various methods such as immunohistochemistry, southern and slot blotting, enzyme immunoassays and fluorescence in situ hybridization have all been employed to evaluate HER-2 gene and protein abnormalities. Of these immunohistochemistry is the most frequently employed but there are valid indications for the other available methods. However, it is prudent that whichever methods employed are standardized, especially those that possess may have a degree of subjectivity in their assessment.

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L3 ANSWER 1 OF 10 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:991469 The Genuine Article (R) Number: 501DB. Bypassing tumor-specific and bispecific antibodies: triggering of antitumor immunity by expression of anti-Fc gamma R scFv on cancer cell surface. Gruel N; Fridman W H; Teillaud J L (Reprint). Ctr Rech Biomed Cordeliers, Unite INSERM 255, 15 Rue Ecole Med, F-75270 Paris 06, France (Reprint); Inst Curie, Lab Biotechnol Anticorps, Paris, France; Inst Curie, Unite INSERM U555, Paris, France. GENE THERAPY (NOV 2001) Vol. 8, No. 22, pp. 1721-1728. Publisher: NATURE PUBLISHING GROUP. HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND. ISSN: 0969-7128. Pub. country: France. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB We have developed a novel immunostimulatory molecule against tumor cells, composed of an anti-Fc gamma RIII (CD16) scFv fused to the platelet-derived growth factor receptor (PDGFR) transmembrane region. This fusion molecule was stably expressed on the tumor cell surface and retained the ability of the parental antibody to bind soluble CD16. Tumor cells expressing anti-CD16 scFv triggered the release of IL-2 by Jurkat-CD16/gamma cells and of TNF alpha by monocytes when co-cultured with these cells. Furthermore, NK cells could kill scFv-transfected HLA(+) class I H1299 lung carcinoma tumor cells, but not the parental cells, indicating that anti-CD16 scFv tumor expression prevents the killer inhibitory receptor (KIR)-mediated inhibition of NK cell cytotoxicity. This anti-CD16 scFv tumor expression also enhanced tumor phagocytosis by IFN gamma -activated macrophages, a mechanism known to induce a protective long-term adaptative immunity to tumors. In vivo Winn tests performed in SCID mice showed that the expression of anti-CD16 scFv on tumor cells, but not of the negative control anti-**phOx** scFv, prevented tumor cell growth. Thus, expression of FcR antibodies or other FcR-specific ligands on tumor cells represents a novel and potent antibody-based gene therapy approach, which may have clinical applications in cancer therapy.

L3 ANSWER 2 OF 10 MEDLINE DUPLICATE 1
2001396197 Document Number: 21235458. PubMed ID: 11337034. A novel retroviral vector that allows the magnetic selection of infected cells. Zhang J; Sapp C M. (Department of Microbiology and Immunology, Markey Cancer Center, University of Kentucky, Lexington, KY40536-0096, USA.. jzhanl@pop.uky.edu) . JOURNAL OF VIROLOGICAL METHODS, (2001 May) 94 (1-2) 1-6. Journal code: HQR; 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.
AB Retroviral vectors are used widely in research and are also being designed

for use in gene therapy trials. In practice, these vectors usually contain a marker gene, which is often a drug selection gene. In this report, a novel retroviral vector has been constructed which contains a gene that allows selection for infected cells by a magnet. This gene is a **single-chain antibody** (sFv) to a specific hapten molecule 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (**phOx**). sFv specific for **phOx** is displayed on the surface of infected cells. This feature allows binding to **phOx**-BSA coated magnetic beads which are used to isolate the infected cells.

L3 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2002 ACS

1999:659579 Document No. 131:283619 Methods and reagents for targeting organic compounds to selected cellular locations. Farinas, Javier (The Regents of the University of California, USA). PCT Int. Appl. WO 9951986 A1 19991014, 69 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US7847 19990408. PRIORITY: US 1998-81118 19980408; US 1998-81340 19980409.

AB The present invention provides methods and reagents for targeting probes to selected cellular locations, through the expression of specific binding partners to that probe within the cell. In one embodiment, the probes may comprise spectroscopic probes that can be used in a method for localizing a specific binding partner within a cell, and for creating assays for post-translational activities. The invention allows the monitoring of the location of such intracellular specific binding partners over time and in response to stimuli, such as test chems. The spectroscopic probes can be used for screening a test chem. for activity. The present invention also includes cells and transgenic organisms comprising the intracellular specific binding partner, wherein the specific binding partner can bind with the spectroscopic probe/ligand conjugate. CHO cells were transfected with cDNAs encoding **single chain antibody** (sFv) fusion products with a Golgi-targeting human .beta.-1,4-galactosyltransferase fragment. The sFv bound to hapten 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (**phOx**)-fluorescein conjugate. The Golgi-targeted **phOx**-fluorescein was used to detect continuous changes in lumenal pH in individual cells.

L3 ANSWER 4 OF 10 MEDLINE DUPLICATE 2

1999175123 Document Number: 99175123. PubMed ID: 10075643. Receptor-mediated targeting of fluorescent probes in living cells. Farinas J; Verkman A S. (Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0521, USA.. javier@itsa.ucsf.edu) . JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Mar 19) 274 (12) 7603-6. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A strategy was developed to label specified sites in living cells with a wide selection of fluorescent or other probes and applied to study pH regulation in Golgi. cDNA transfection was used to target a **single-chain antibody** to a specified site such as an organelle lumen. The targeted antibody functioned as a high affinity receptor to trap cell-permeable hapten-fluorophore conjugates. Synthesized conjugates of a hapten (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one, **phOx**) and fluorescent probes (Bodipy Fl, tetramethylrhodamine, fluorescein) were bound with high affinity (approximately 5 nM) and specific localization to the **single-chain antibody** expressed in the endoplasmic reticulum, Golgi, and plasma membrane of living Chinese hamster ovary cells. Using the pH-sensitive **phOx**-fluorescein conjugate and ratio imaging microscopy, pH was measured in the lumen of Golgi (pH 6.25 +/- 0.06). Measurements of pH-dependent vacuolar H+/ATPase pump activity and H+ leak in Golgi

provided direct evidence that resting Golgi pH is determined by balanced leak-pump kinetics rather than the inability of the H+/ATPase to pump against an electrochemical gradient. Like expression of the green fluorescent protein, the receptor-mediated fluorophore targeting approach permits specific intracellular fluorescence labeling. A significant advantage of the new approach is the ability to target chemical probes with custom-designed spectral and indicator properties.

L3 ANSWER 5 OF 10 MEDLINE DUPLICATE 3
1999284107 Document Number: 99284107. PubMed ID: 10357215. T cell activation by monoclonal antibodies bound to tumor cells by a cell surface displayed **single-chain antibody**. Rode H J; Moebius U; Little M. (Diagnostics and Experimental Therapy Programme, German Cancer Research Center, Heidelberg.) JOURNAL OF IMMUNOLOGICAL METHODS, (1999 Apr 22) 224 (1-2) 151-60. Journal code: IFE; 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Tumor cells often lack the costimulatory molecules necessary for T cell activation. However, the transformation of cells with more than one stimulatory molecule is a difficult procedure. We therefore developed a retroviral vector for the expression of a cell membrane anchored **single-chain antibody** fragment (scFv) directed against the hapten 4-ethoxymethylene-2-phenyl-2-oxazoline-5-one (**phOx**). Proteins and peptides can be readily modified with this hapten, thus, enabling them to be bound to cells with the cell surface displayed anti-**phOx** scFv. To test combinations of surface-bound stimulatory molecules on T cell activation, SK-Mel63 human melanoma cells expressing the membrane anchored anti-**phOx** scFv were incubated with **phOx**-labeled mAbs against CD3, CD28 and CD5. Cells presenting a given mixture of modified anti-CD3 and anti-CD28 molecules stimulated T cell activation better than any single antibody and a given mixture of anti-CD3, anti-CD28 and anti-CD5 provided a stimulatory response higher than the best double combination. However, the relative concentrations are very important and must be carefully chosen. Concentrations of antibodies giving good T cell responses when used alone can block synergistic effects.

L3 ANSWER 6 OF 10 MEDLINE DUPLICATE 4
97158585 Document Number: 97158585. PubMed ID: 9005945. High level production of soluble **single chain antibodies** in small-scale Escherichia coli cultures. Kipriyanov S M; Moldenhauer G; Little M. (Diagnostics and Experimental Therapy Programme, German Cancer Research Center, Heidelberg.) JOURNAL OF IMMUNOLOGICAL METHODS, (1997 Jan 15) 200 (1-2) 69-77. Journal code: IFE; 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB We have investigated the effect of growth and induction conditions on the production of soluble single-chain Fv antibody fragments in Escherichia coli under the control of wt lac promoter. The scFv was directed into the periplasmic space by a pelB leader sequence. Addition of sucrose to the medium gave a 15-25-fold increase in the yield of soluble scFv-**phOx** (3.0 mg/l) for bacterial shake-tube cultures and an increase of 80-150-fold (16.5 mg/l) for shake-flask cultures. Using flask culture in the presence of 0.4 M sucrose, a significant amount of scFv was released into the medium. We found that the scFv could be made to accumulate in the periplasm or be secreted into the medium by simply changing the incubation conditions and the concentration of the inducer. The ratio between soluble antibody fragments and insoluble scFv aggregates proved to be dependent on the strength of the promoter. Lowering the incubation temperature below 20 degrees C had no effect on the yield of soluble antibody fragments in the periplasm, but they were no longer secreted into the medium. An example of high level production in shake-flask cultures and one-step purification by immobilized metal affinity chromatography (IMAC) is described for a soluble scFv specific for the T cell surface antigen CD3. The biological activity of the purified anti-CD3 scFv was demonstrated by flow cytometry. This method should be especially useful for the functional screening of a large number

of clones in small-scale cultures.

L3 ANSWER 7 OF 10 MEDLINE DUPLICATE 5
97046295 Document Number: 97046295. PubMed ID: 8891216. Cell surface display of a **single-chain antibody** for attaching polypeptides. Rode H J; Little M; Fuchs P; Dorsam H; Schooltink H; de Ines C; Dubel S; Breitling F. (German Cancer Research Center, Heidelberg, Germany.) BIOTECHNIQUES, (1996 Oct) 21 (4) 650, 652-3, 655-6, 658. Journal code: AN3; 8306785. ISSN: 0736-6205. Pub. country: United States. Language: English.

AB To provide an efficient means of coupling proteins, peptides and other suitable moieties to cells, we have constructed a retroviral expression vector for cell surface display of a **single-chain antibody** (scFv) against the hapten 4-ethoxymethylene-2-phenyl-oxazo-line-5-one (**ph0x**). The hapten **ph0x** can be easily conjugated to primary amino and sulfhydryl groups, thus providing points of attachment for the cell surface-bound anti-**ph0x** scFv. This universal cell coupling system could prove to be particularly useful for anchoring monoclonal antibodies for tumor targeting and to present co-stimulatory molecules and other ligands (even mixtures) at the cell surface for gene therapy.

L3 ANSWER 8 OF 10 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 6
96305483 EMBASE Document No.: 1996305483. Cell surface display of a **single-chain antibody** for attaching polypeptides. Rode H.-J.; Little M.; Fuchs P.; Dorsam H.; Schooltink H.; De Ines C.; Dubel S.; Breitling F.. Recombinant Antibody Research Unit, German Cancer Research Center, Im Neuenheimer Feld 280,69120 Heidelberg, Germany. BioTechniques 21/4 (650-658) 1996.
ISSN: 0736-6205. CODEN: BTNQDO. Pub. Country: United States. Language: English. Summary Language: English.

AB To provide an efficient means of coupling proteins, peptides and other suitable moieties to cells, we have constructed a retroviral expression vector for cell surface display of a **single-chain antibody** (scFv) against the hapten 4-ethoxymethylene-2-phenyl-oxazoline-5-one (**ph0x**). The hapten **ph0x** can be easily conjugated to primary amino and sulfhydryl groups, thus providing points of attachment for the cell surface-bound anti-**ph0x** scFv. This universal cell coupling system could prove to be particularly useful for anchoring monoclonal antibodies for tumor targeting and to present co-stimulator molecules and other ligands (even mixtures) at the cell surface for gene therapy.

L3 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2002 ACS
1995:764961 Document No. 123:307516 A single expression system for the display, purification and conjugation of **single-chain antibodies**. Hayashi, Nakanobu; Kipriyanov, Sergey; Fuchs, Patrick; Welschof, Martin; Doersam, Heinz; Little, Melvyn (Recombinant Antibody Group, German Cancer Research Center, Heidelberg, 69120, Germany). Gene, 160(1), 129-30 (English) 1995. CODEN: GENED6. ISSN: 0378-1119.

AB To facilitate the purifn. and conjugation of **single-chain antibodies** (scFv) selected from a phage display library, we have incorporated His6, an amber stop codon, and a C-terminal Cys were incorporated into a surface expression vector. The vector also contains a lacIq gene for improving the efficiency of regulation and a sequence coding for a marker peptide.

L3 ANSWER 10 OF 10 MEDLINE DUPLICATE 7
93372090 Document Number: 93372090. PubMed ID: 8364031. Phage display and selection of a site-directed randomized **single-chain antibody** Fv fragment for its affinity improvement. Riechmann L; Weill M. (MRC Laboratory of Molecular Biology, Cambridge, UK.) BIOCHEMISTRY, (1993 Aug 31) 32 (34) 8848-55. Journal code: A0G; 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The affinity of an antibody Fv fragment was improved by semirational design involving site-directed randomization and phage display. On the basis of the predicted model of an anti-2-phenyloxazol-5-one (**phOx**) antibody Fv fragment, into which the ligand was inserted with the help of nuclear Overhauser enhancement (NOE) data, residues close to the hapten were identified. Seven of these residues in the third hypervariable regions of light and heavy chains were randomized in polymerase chain reactions (PCR) using degenerate oligonucleotides. Resulting clones were expressed as single-chain Fv (scFV) fragments on the surface of filamentous phage and selected for binding to **phOx**-conjugated bovine serum albumin. Selected Fv fragments were analyzed for hapten affinity by fluorescence quenching, and several mutants with improved affinities were identified. Phage selection on the basis of binding was very successful when phage scFv mutants differed in affinity by at least a factor of 6. Smaller differences did not result in predominant selection of the best binder. Combination of the two point mutations most crucial for improved hapten binding decreased the dissociation constant of the Fv for **phOx** 11-14-fold. Hapten binding of the improved Fv was analyzed in NOE experiments.

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=> d his

     (FILE 'HOME' ENTERED AT 08:17:45 ON 03 APR 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 08:18:06 ON
     03 APR 2002
L1          3858 S SINGLE CHAIN ANTIBODY
L2            33 S L1 AND "PHOX"
L3            10 DUP REMOVE L2 (23 DUPLICATES REMOVED)

=> s l2 and membrane permeant probe
L4             0 L2 AND MEMBRANE PERMEANT PROBE

=> s "PhOx bodiyp fl"
L5             0 "PHOX BODIYP FL"

=> s "PhOx Bodipy Fl"
L6             0 "PHOX BODIPY FL"

=> s probe
L7        718845 PROBE

=> s l7 and conjugate
L8          6415 L7 AND CONJUGATE

=> s l8 and PhOx
L9             6 L8 AND PHOX

=> dup remove l9
PROCESSING COMPLETED FOR L9
L10            2 DUP REMOVE L9 (4 DUPLICATES REMOVED)

=> d l10 1-2 cbib abs

L10  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2002 ACS
1999:659579   Document No. 131:283619  Methods and reagents for targeting
     organic compounds to selected cellular locations.  Farinas, Javier (The
     Regents of the University of California, USA).  PCT Int. Appl. WO 9951986
     A1 19991014, 69 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA,
     BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH,
     GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
     LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
     SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ,
     MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES,
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FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US7847 19990408. PRIORITY: US 1998-81118 19980408; US 1998-81340 19980409.

AB The present invention provides methods and reagents for targeting **probes** to selected cellular locations, through the expression of specific binding partners to that **probe** within the cell. In one embodiment, the **probes** may comprise spectroscopic **probes** that can be used in a method for localizing a specific binding partner within a cell, and for creating assays for post-translational activities. The invention allows the monitoring of the location of such intracellular specific binding partners over time and in response to stimuli, such as test chems. The spectroscopic **probes** can be used for screening a test chem. for activity. The present invention also includes cells and transgenic organisms comprising the intracellular specific binding partner, wherein the specific binding partner can bind with the spectroscopic **probe**/ligand **conjugate**. CHO cells were transfected with cDNAs encoding single chain antibody (sFv) fusion products with a Golgi-targeting human .beta.-1,4-galactosyltransferase fragment. The sFv bound to hapten 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (**phOx**)-fluorescein **conjugate**. The Golgi-targeted **phOx**-fluorescein was used to detect continuous changes in lumenal pH in individual cells.

L10 ANSWER 2 OF 2 MEDLINE DUPLICATE 1

1999175123 Document Number: 99175123. PubMed ID: 10075643.

Receptor-mediated targeting of fluorescent **probes** in living cells. Farinas J; Verkman A S. (Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0521, USA.. javier@itsa.ucsf.edu) . JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Mar 19) 274 (12) 7603-6. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A strategy was developed to label specified sites in living cells with a wide selection of fluorescent or other **probes** and applied to study pH regulation in Golgi. cDNA transfection was used to target a single-chain antibody to a specified site such as an organelle lumen. The targeted antibody functioned as a high affinity receptor to trap cell-permeable hapten-fluorophore **conjugates**. Synthesized **conjugates** of a hapten (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one, **phOx**) and fluorescent **probes** (Bodipy Fl, tetramethylrhodamine, fluorescein) were bound with high affinity (approximately 5 nM) and specific localization to the single-chain antibody expressed in the endoplasmic reticulum, Golgi, and plasma membrane of living Chinese hamster ovary cells. Using the pH-sensitive **phOx**-fluorescein **conjugate** and ratio imaging microscopy, pH was measured in the lumen of Golgi (pH 6.25 +/- 0.06). Measurements of pH-dependent vacuolar H+/ATPase pump activity and H+ leak in Golgi provided direct evidence that resting Golgi pH is determined by balanced leak-pump kinetics rather than the inability of the H+/ATPase to pump against an electrochemical gradient. Like expression of the green fluorescent protein, the receptor-mediated fluorophore targeting approach permits specific intracellular fluorescence labeling. A significant advantage of the new approach is the ability to target chemical **probes** with custom-designed spectral and indicator properties.

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=> s Farinas j?/au
L11        146 FARINAS J?/AU

=> s l11 and intracellular fluorescent probe
L12          0 L11 AND INTRACELLULAR FLUORESCENT PROBE

=> s l11 and single chain antibody
L13          6 L11 AND SINGLE CHAIN ANTIBODY

=> dup remove l13
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PROCESSING COMPLETED FOR L13
L14 2 DUP REMOVE L13 (4 DUPLICATES REMOVED)

=> d l14 1-2 cbib abs

L14 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
1999:659579 Document No. 131:283619 Methods and reagents for targeting organic compounds to selected cellular locations. **Farinas, Javier** (The Regents of the University of California, USA). PCT Int. Appl. WO 9951986 A1 19991014, 69 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US7847 19990408. PRIORITY: US 1998-81118 19980408; US 1998-81340 19980409.

AB The present invention provides methods and reagents for targeting probes to selected cellular locations, through the expression of specific binding partners to that probe within the cell. In one embodiment, the probes may comprise spectroscopic probes that can be used in a method for localizing a specific binding partner within a cell, and for creating assays for post-translational activities. The invention allows the monitoring of the location of such intracellular specific binding partners over time and in response to stimuli, such as test chems. The spectroscopic probes can be used for screening a test chem. for activity. The present invention also includes cells and transgenic organisms comprising the intracellular specific binding partner, wherein the specific binding partner can bind with the spectroscopic probe/ligand conjugate. CHO cells were transfected with cDNAs encoding **single chain antibody** (sFv) fusion products with a Golgi-targeting human .beta.-1,4-galactosyltransferase fragment. The sFv bound to hapten 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (phOx)-fluorescein conjugate. The Golgi-targeted phOx-fluorescein was used to detect continuous changes in lumenal pH in individual cells.

L14 ANSWER 2 OF 2 MEDLINE DUPLICATE 1
1999175123 Document Number: 99175123. PubMed ID: 10075643.
Receptor-mediated targeting of fluorescent probes in living cells. **Farinas J**; Verkman A S. (Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0521, USA.. javier@itsa.ucsf.edu) . JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Mar 19) 274 (12) 7603-6. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A strategy was developed to label specified sites in living cells with a wide selection of fluorescent or other probes and applied to study pH regulation in Golgi. cDNA transfection was used to target a **single -chain antibody** to a specified site such as an organelle lumen. The targeted antibody functioned as a high affinity receptor to trap cell-permeable hapten-fluorophore conjugates. Synthesized conjugates of a hapten (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one, phOx) and fluorescent probes (Bodipy Fl, tetramethylrhodamine, fluorescein) were bound with high affinity (approximately 5 nM) and specific localization to the **single-chain antibody** expressed in the endoplasmic reticulum, Golgi, and plasma membrane of living Chinese hamster ovary cells. Using the pH-sensitive phOx-fluorescein conjugate and ratio imaging microscopy, pH was measured in the lumen of Golgi (pH 6.25 +/- 0.06). Measurements of pH-dependent vacuolar H+/ATPase pump activity and H+ leak in Golgi provided direct evidence that resting Golgi pH is determined by balanced leak-pump kinetics rather than the inability of the H+/ATPase to pump against an electrochemical gradient. Like expression of the green fluorescent protein, the receptor-mediated fluorophore targeting approach permits specific intracellular fluorescence labeling. A significant advantage of the new approach is the ability to target

chemical probes with custom-designed spectral and indicator properties.

=> s intracellular targeting
L15 1400 INTRACELLULAR TARGETING

=> s l15 and single chain
L16 24 L15 AND SINGLE CHAIN

=> s l16 and probe
L17 0 L16 AND PROBE

=> dup remove l16
PROCESSING COMPLETED FOR L16
L18 11 DUP REMOVE L16 (13 DUPLICATES REMOVED)

=> d l18 1-11 cbib abs

L18 ANSWER 1 OF 11 MEDLINE DUPLICATE 1
2000040151 Document Number: 20040151. PubMed ID: 10571661. Ras and p53 **intracellular targeting** with recombinant **single -chain** Fv (scFv) fragments: a novel approach for cancer therapy?. Cochet O; Gruel N; Fridman W H; Teillaud J L. (Laboratoire de Biotechnologie des Anticorps, Paris, France.) CANCER DETECTION AND PREVENTION, (1999) 23 (6) 506-10. Ref: 13. Journal code: CNZ; 7704778. ISSN: 0361-090X. Pub. country: United States. Language: English.
AB Intracellular expression of recombinant antibodies allows one to interfere with the functions of oncogenic molecules expressed in various cell compartments and has therefore a vast clinical potential in cancer therapy. We inhibited the functions of oncogenic Ras mutant forms by intracellular expression of a neutralizing **single-chain** antibody (scFv). In vitro studies indicated that the scFv is expressed in the cytosol of Xenopus laevis oocytes and of tumor cells, blocks ras-mediated activation processes, and induces tumor cell death. In vivo studies performed using scFv cDNA inserted into an adenoviral vector showed that the scFv dramatically affects tumor growth. Second, intracellular expression of scFvs directed against p53 indicated that these antibody fragments can be successfully targeted to cell nucleus, bind p53, and partially restore the transcriptional activity of p53 mutants in human tumor cells. Thus, intracellular scFvs directed against oncogenic molecules may represent a new class of antitumor agents.

L18 ANSWER 2 OF 11 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 2
2000213174 EMBASE Extended half-life and elevated steady-state level of a **single-chain** Fv intrabody are critical for specific intracellular retargeting of its antigen, caspase-7. Zhu Q.; Zeng C.; Huhalov A.; Yao J.; Turi T.G.; Danley D.; Hynes T.; Cong Y.; DiMattia D.; Kennedy S.; Daumy G.; Schaeffer E.; Marasco W.A.; Huston J.S.. Q. Zhu, IntraImmune Therapies Inc., P.O. Box 15599, Boston, MA 02215-0011, United States. quanzhu@tiac.net. Journal of Immunological Methods 231/1-2 (207-222) 1999.
Refs: 47.
ISSN: 0022-1759. CODEN: JIMMBG.
Publisher Ident.: S 0022-1759(99)00158-1. Pub. Country: Netherlands.
Language: English. Summary Language: English.
AB Two **single-chain** Fv (sFv) antibodies (C8 and H2) specific for Mch3/caspase-7, a component in the signaling pathway for induction of apoptosis, were genetically fused to different **intracellular targeting** signals and analyzed by expression in mammalian cells. Immunofluorescence microscopy confirmed that these anti-caspase-7 intrabodies were expressed in the cellular compartments dictated by their C-terminal trafficking signals. Cytosolic caspase-7 was successfully retargeted to different subcellular compartments by specific intrabodies through direct association of antigen with intrabody. Sequestration of caspase-7 in nuclei had a significant

biological impact in that the expression of a nuclear-targeted anti-caspase-7 intrabody in a stable Jurkat cell line markedly inhibited staurosporine- induced apoptosis. The criteria for choosing an optimal intrabody were also evaluated in this study. A gene dosage titration study demonstrated that the C8 intrabody was more potent in retargeting of caspase-7 than the H2 intrabody, even though the H2 sFv had a higher affinity for caspase-7 than the C8. Pulse-chase experiments and western blot analysis revealed that the anti-caspase-7 C8 sFv intrabodies exhibited a long half-life (> 8 h) and high steady-state levels of protein accumulation, while the H2 intrabodies had a half-life of 2 h and less protein at steady state. These results suggest that the choice of sFv as an intrabody depends critically on the intracellular sFv protein having an extended half-life and elevated steady-state level. Thus, extended half-life must be considered together with sFv antibody specificity and affinity when choosing an optimal sFv intrabody for functional studies of cellular proteins. (C) 1999 Elsevier Science B.V.

L18 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2002 ACS
1999:326531 Document No. 131:212865 Intracellular and cell surface displayed **single-chain** diabodies. Kontermann, Roland E.; Muller, Rolf (Emil-Mannkopff-Strasse 2, Institut fur Molekularbiologie und Tumorforschung, Philipps-Universitat Marburg, Marburg, D-35033, Germany). J. Immunol. Methods, 226(1-2), 179-188 (English) 1999. CODEN: JIMMBG. ISSN: 0022-1759. Publisher: Elsevier Science B.V..

AB Intracellularly expressed antibody fragments have found various applications in therapy by virtue of their ability to inhibit the function of cellular proteins or interfere with subcellular trafficking. Bivalent antibody fragments might further improve this inhibitory potential by increasing the functional affinity and bispecific antibody fragments may also be useful for the intracellular retargeting of mols. Here, the authors have evaluated the functional expression of intracellular diabodies. A previously constructed secreted bispecific **single-chain** diabody directed against carcinoembryonic antigen and Escherichia coli .beta.-galactosidase was modified for subcellular targeting to the cell surface membrane, endoplasmic reticulum, mitochondria, cytoplasm, and nucleus. Subcellular localization was analyzed by immunofluorescence, and the assembly of functional antibodies was analyzed by binding of .beta.-galactosidase to the antibody fragment and subsequent substrate conversion. Bispecific **single-chain** diabodies could be directed to all subcellular compartments analyzed. However, functional assembly was only obsd. for **single-chain** diabodies retained in the endoplasmic reticulum or displayed in the cell membrane while no antigen binding activity was seen with diabodies directed to the cytoplasm, nucleus, or mitochondria. The results demonstrate the functional expression of bispecific recombinant antibody fragments in the secretory pathway and integration into the plasma membrane of mammalian cells.

L18 ANSWER 4 OF 11 MEDLINE DUPLICATE 3
1998391083 Document Number: 98391083. PubMed ID: 9725267. cDNA encoding a **single-chain** antibody to HIV p17 with cytoplasmic or nuclear retention signals inhibits HIV-1 replication. Tewari D; Goldstein S L; Notkins A L; Zhou P. (Oral Infection and Immunity Branch, National Institute of Dental Research, Bethesda, MD 20892, USA.) JOURNAL OF IMMUNOLOGY, (1998 Sep 1) 161 (5) 2642-7. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB HIV-1 gag p17 protein is an attractive target for molecular intervention, because it is involved in the viral replication cycle at both the pre- and postintegration levels. In the present experiments, we targeted p17 by intracellularly expressing a cDNA encoding an Ab to p17. cDNA from a hybridoma-secreting Ab to p17 was cloned, sequenced, reconstructed as a **single-chain** Ab fragment (scFv), and expressed in the cytoplasm or nucleus with appropriate retention signals. The expressed scFvs had no effect on T cell growth or CD4 expression and bound specifically to HIV-1 p17. Human CD4+ Jurkat T cells that expressed scFvs

and were infected with HIV-1 showed a marked reduction in virus replication compared with cells expressing vector alone. The inhibition of virus replication was more pronounced when scFvs were expressed in the cytoplasm rather than the nucleus. From these studies, we conclude that the intracellular expression of a **single-chain** Ab to p17 inhibits HIV replication; in addition, the degree of inhibition is related to the **intracellular targeting** site.

L18 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2002 ACS
1998:792833 Document No. 130:195381 **Intracellular targeting** of oncogenes: a novel approach for cancer therapy. Cochet, Olivier; Delumeau, Isabelle; Kenigsberg, Mireille; Gruel, Nadege; Schweighoffer, Fabien; Bracco, Laurent; Teillaud, Jean Luc; Tocque, Bruno (Lab. Biotechnol. Anticorps, Inst. Curie, Paris, Fr.). Intrabodies, 129-146. Editor(s): Marasco, Wayne A. Springer: Berlin, Germany. (English) 1998. CODEN: 67ASA9.

AB A review with 41 refs. This paper discusses Ras as a target for cancer treatment, the generation and in vitro characterization of scFvs targeted to Ras, and the functional activity of the anti-Ras scFvs in Xenopus oocytes and mammalian cells.

L18 ANSWER 6 OF 11 MEDLINE DUPLICATE 4
1998046010 Document Number: 98046010. PubMed ID: 9384607. Biosynthesis and **intracellular targeting** of the CLN3 protein defective in Batten disease. Jarvela I; Sainio M; Rantamaki T; Olkkonen V M; Carpen O; Peltonen L; Jalanko A. (National Public Health Institute, Department of Human Molecular Genetics, Mannerheimintie 166, 00300 Helsinki, Finland.. irma.jarvela@ktl.fi) . HUMAN MOLECULAR GENETICS, (1998 Jan) 7 (1) 85-90. Journal code: BRC; 9208958. ISSN: 0964-6906. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Batten disease (juvenile-onset neuronal ceroid lipofuscinosis, JNCL), the most common neurodegenerative disorder of childhood, is caused by mutations in a recently identified gene (CLN3) localized to chromosome 16p11.2-12.1. To elucidate the biosynthesis and localization of the CLN3 protein, we expressed CLN3 cDNA in COS-1 and HeLa cell lines. In vitro translation, immunoprecipitation and Western blotting analyses detected an approximately 43 kDa polypeptide. Pulse-chase experiments indicated that the CLN3 protein is synthesized as an N -glycosylated **single-chain** polypeptide, which was not detected in growth medium. Confocal immunofluorescence microscopy revealed that the CLN3 protein is localized to the lysosomal compartment. These results provide evidence that Batten disease can be classified as a member of lysosomal diseases.

L18 ANSWER 7 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
97:758630 The Genuine Article (R) Number: YA164. Inhibition of murine leukaemia virus retrotranscription by the intracellular expression of a phage-derived anti-reverse transcriptase antibody fragment. Gargano N; Cattaneo A (Reprint). MRC, MOL BIOL LAB, HILLS RD, CAMBRIDGE CB2 2QH, ENGLAND (Reprint); MRC, MOL BIOL LAB, CAMBRIDGE CB2 2QH, ENGLAND. JOURNAL OF GENERAL VIROLOGY (OCT 1997) Vol. 78, Part 10, pp. 2591-2599. Publisher: SOC GENERAL MICROBIOLOGY. MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING, BERKS, ENGLAND RG7 1AE. ISSN: 0022-1317. Pub. country: ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **intracellular targeting** of recombinant antibodies is an experimental strategy to interfere with the function of selected molecules that is being utilized in a variety of different systems for research and medical applications. Since recombinant antibodies are increasingly being derived from phage display libraries, we have exploited phage technology to isolate, from a large combinatorial library, human antibody fragments directed against human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT). We describe in this paper the in vitro and in vivo properties of a neutralizing anti-RT antibody fragment, We demonstrate that the heavy chain domain (VH-CH1) of the phage-derived antibody is able to inhibit the retroviral enzyme, in that

it neutralizes the RNA-dependent DNA polymerase activity of HIV-1 RT. The VH-CH1 antibody fragment also neutralizes the activity of RT of drug-resistant HIV-1 mutants as well as that of murine retrovirus RT. To confirm the broad reactivity of the synthetic antibody fragment, we have assessed the ability of the intracellularly expressed VH-CH1 protein to interfere with murine retroviral infection, To this end, we developed an in vivo selection procedure based on the antibody-mediated resistance to a cytotoxic retrovirus and used this selection procedure to rescue, from a heterogeneous population, cells expressing the VH-CH1 antibody fragment. We finally demonstrate that the intracellular expression of the recombinant heavy chain antibody fragment leads to an efficient inhibition of viral retrotranscription by murine-based retrovirus.

L18 ANSWER 8 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
96:862098 The Genuine Article (R) Number: VT185. Intracellular antibody technique and its medical application.. Zhou C S (Reprint); Zhen Y S. CHINESE ACAD MED SCI, PEKING UNION MED COLL, INST MED BIOTECHNOL, BEIJING 100050, PEOPLES R CHINA. PROGRESS IN BIOCHEMISTRY AND BIOPHYSICS (OCT 1996) Vol. 23, No. 5, pp. 386-390. Publisher: SCIENCE PRESS. 16 DONGHUANGCHENGGEN NORTH ST, BEIJING 100707, PEOPLES R CHINA. ISSN: 1000-3282. Pub. country: PEOPLES R CHINA. Language: Chinese.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Intracellular antibody refers to the recombinant antibody expressed intracellularly. **Single-chain** Fv fragment, one common form of intracellular antibody with high affinity, can be expressed in transfected nonlymphocytes and targets to a particular cellular compartment to interfere the activity of some macromolecular substances or the process of their secretion. Intracellular antibody has been proved to be capable of inhibiting growth factor receptors, inactivating oncoproteins and inhibiting HIV-1 replication. Intracellular antibody technique is a novel gene therapy approach with potentiality in medical application.

L18 ANSWER 9 OF 11 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:139183 Document No.: PREV199799438386. **Intracellular targeting** by anti-p21-ras scFv: A model for anti-tumor therapy. Teillaud, Jean-Luc (1); Delumeau, Isabelle; Schweighoffer, Fabien; Cochet, Olivier (1); Janicot, Michel; Kenigsberg, Mireille; Tocque, B.. (1) Lab. Biotechnol. Anticorps, Paris France. Immunotechnology (Amsterdam), (1996) Vol. 2, No. 4, pp. 306. Meeting Info.: 1996 Keystone Meeting on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites Taos, New Mexico, USA February 22-28, 1996 ISSN: 1380-2933. Language: English.

L18 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2002 ACS
1996:196516 Document No. 124:254430 Glycosylation and phosphorylation of lysosomal glycosylasparaginase. Park, Hyejeong; Vettese-Dadey, Michelle; Aronson, Nathan N., Jr. (Dep. Biochemistry Molecular Biology, Univ. South Alabama, Mobile, AL, 36688, USA). Arch. Biochem. Biophys., 328(1), 73-7 (English) 1996. CODEN: ABBIA4. ISSN: 0003-9861.
AB Glycosylasparaginase (EC 3.5.1.26) is a lysosomal amidase which hydrolyzes the bond between asparagine and the sugar moiety in N-linked glycoproteins. Deficiency of the enzyme results in aspartylglycosaminuria (AGU), the most common disorder of glycoprotein degrdn. Mature enzyme is formed by two proteolytic cleavage steps subsequent to removal of the signal peptide: (1) an activation cleavage, in the ER, of the initial **single-chain** 49-kDa polypeptide into a 27-kDa .alpha.- and 19-kDa .beta.-subunit; (2) a cleavage, in the lysosome, which removes 10 amino acids from the C-terminus of the .alpha.-subunit without affecting enzyme activity. Each subunit of glycosylasparaginase contains one N-linked oligosaccharide (N38, .alpha.-subunit; N308, .beta.-subunit). Both oligosaccharides were phosphorylated and releasable by Endo-H digestion, indicating they were of the high-mannose type. These glycosylation sequenons were mutagenized to det. the role of the oligosaccharide at each site in proper folding and transport of glycosylasparaginase. An N38D mutant underwent the lysosomal processing

step, indicating that targeting to lysosomes can be via the phosphorylated .beta.-subunit oligosaccharide alone. Deletion of the .beta.-subunit oligosaccharide at N308 by an aspartic acid substitution resulted in very little protein or enzyme activity in transfected cells, reemphasizing that glycosylation of the .beta.-subunit site is important for efficient folding and/or targeting. A different mutation to eliminate the same N-glycosylation sequenon (T310A) yielded more protein and enzyme activity, and a double mutant N38D/T310A yielded the same results as the single .beta.-subunit substitution. Yield of enzyme for all mutants was increased in cells treated with brefeldin A. The N308 glycosylation site of the .beta.-subunit appears to be more important in maintaining normal transport and stability of human glycosylasparaginase.

L18 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2002 ACS
1993:119859 Document No. 118:119859 Expression of mouse cathepsin L cDNA in Saccharomyces cerevisiae: evidence that cathepsin L is sorted for targeting to yeast vacuole. Nishimura, Yukio; Kato, Keitaro (Fac. Pharm. Sci., Kyushu Univ., Fukuoka, 812, Japan). Arch. Biochem. Biophys., 298(2), 318-24 (English) 1992. CODEN: ABBIA4. ISSN: 0003-9861.

AB To investigate the intracellular transport mechanism of lysosomal cathepsin L in yeast cells, mouse cathepsin L was expressed in S. cerevisiae by placing the coding region under the control of the promoter of the yeast glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene. Immunoblotting anal. with an antibody specific for rat cathepsin L revealed that yeast cells carrying the cathepsin L coding sequence produced 39- and 30-kDa products, which correspond to rat procathepsin L and the **single-chain** form of mature cathepsin L, resp. The precursor polypeptide showed sensitivity toward endoglycosidase H treatment. Cell fractionation expts. demonstrated that the processed form of 30-kDa cathepsin L was colocalized to the yeast vacuole with the marker enzyme carboxypeptidase Y in a Ficoll step gradient. In the prepd. vacuolar fraction, a considerable amt. of cathepsin L cofractionated with the vacuolar membranes. Furthermore, phase sepn. expts. with Triton X-114 provided the first evidence showing that the mature form of cathespin L polypeptide is strongly assocd. with the vacuolar membranes. Therefore, the present results suggest that the mouse cathepsin L precursor is initially synthesized as the proenzyme in yeast cells and then correctly delivered to the vacuole. During the intracellular sorting pathway, procathepsin L undergoes post-translational proteolytic processing to generate the mature enzyme. Based on these lines of evidence, it is proposed that cathepsin L is recognized by mechanisms similar to those for the intracellular sorting and processing of vacuolar proteins in the yeast cells.

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(FILE 'HOME' ENTERED AT 08:17:45 ON 03 APR 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 08:18:06 ON 03 APR 2002

L1	3858	S SINGLE CHAIN ANTIBODY
L2	33	S L1 AND "PHOX"
L3	10	DUP REMOVE L2 (23 DUPLICATES REMOVED)
L4	0	S L2 AND MEMBRANE PERMEANT PROBE
L5	0	S "PHOX BODIYP FL"
L6	0	S "PHOX BODIPY FL"
L7	718845	S PROBE
L8	6415	S L7 AND CONJUGATE
L9	6	S L8 AND PHOX
L10	2	DUP REMOVE L9 (4 DUPLICATES REMOVED)
L11	146	S FARINAS J?/AU
L12	0	S L11 AND INTRACELLULAR FLUORESCENT PROBE
L13	6	S L11 AND SINGLE CHAIN ANTIBODY
L14	2	DUP REMOVE L13 (4 DUPLICATES REMOVED)

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L15         1400 S INTRACELLULAR TARGETING
L16           24 S L15 AND SINGLE CHAIN
L17            0 S L16 AND PROBE
L18           11 DUP REMOVE L16 (13 DUPLICATES REMOVED)

=> dup remove l11
PROCESSING COMPLETED FOR L11
L19           70 DUP REMOVE L11 (76 DUPLICATES REMOVED)

=> s l19 and "PhOx conjugate"
L20            0 L19 AND "PHOX CONJUGATE"

=> s l19 and ligand conjugate
L21            1 L19 AND LIGAND CONJUGATE

=> d l21 cbib abs

L21  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2002 ACS
1999:659579   Document No. 131:283619  Methods and reagents for targeting
     organic compounds to selected cellular locations.  Farinas, Javier
     (The Regents of the University of California, USA).  PCT Int. Appl. WO
     9951986 A1 19991014, 69 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU,
     AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD,
     GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
     LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG,
     SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY,
     KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE,
     DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN,
     TD, TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1999-US7847 19990408.
     PRIORITY: US 1998-81118 19980408; US 1998-81340 19980409.
AB   The present invention provides methods and reagents for targeting probes
     to selected cellular locations, through the expression of specific binding
     partners to that probe within the cell.  In one embodiment, the probes may
     comprise spectroscopic probes that can be used in a method for localizing
     a specific binding partner within a cell, and for creating assays for
     post-translational activities.  The invention allows the monitoring of the
     location of such intracellular specific binding partners over time and in
     response to stimuli, such as test chems.  The spectroscopic probes can be
     used for screening a test chem. for activity.  The present invention also
     includes cells and transgenic organisms comprising the intracellular
     specific binding partner, wherein the specific binding partner can bind
     with the spectroscopic probe/ligand conjugate.  CHO
     cells were transfected with cDNAs encoding single chain antibody (sFv)
     fusion products with a Golgi-targeting human .beta.-1,4-
     galactosyltransferase fragment.  The sFv bound to hapten
     4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (phOx)-fluorescein conjugate.
     The Golgi-targeted phOx-fluorescein was used to detect continuous changes
     in lumenal pH in individual cells.


=> s spectroscopic probe
L22         1533 SPECTROSCOPIC PROBE

=> s l22 and cell permeant
   3 FILES SEARCHED...
L23            0 L22 AND CELL PERMEANT

=> s l22 and Bodipy Fl
L24            0 L22 AND BODIPY FL

=> s cell expressing single chain antibody
   2 FILES SEARCHED...
   4 FILES SEARCHED...
L25            1 CELL EXPRESSING SINGLE CHAIN ANTIBODY
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=> d l25 cbib abs

L25 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
2000:450926 Document No. 134:84694 Prostate-specific membrane antigen (PSMA)-specific monoclonal antibodies in the treatment of prostate and other cancers. Gong, Michael C.; Chang, Sam S.; Sadelain, Michel; Bander, Neil H.; Heston, Warren D. W. (Department of Urology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA). Cancer and Metastasis Reviews, Volume Date 1999, 18(4), 483-490 (English) 2000. CODEN: CMRED4. ISSN: 0167-7659. Publisher: Kluwer Academic Publishers.
AB A review with 35 refs. Prostate-specific membrane antigen (PSMA) is a cell surface glycoprotein that is expressed by prostate epithelial cells. PSMA-specific monoclonal antibodies have been utilized to characterize the biol. function and in vivo biodistribution of PSMA. PSMA is an attractive target protein for monoclonal antibody directed imaging or therapeutics for prostate cancer since its expression is relatively restricted to prostate epithelial cells and is over-expressed in prostate cancer, including in advanced stages. Currently, clin. usage of PSMA specific monoclonal antibodies has been limited to diagnostic immunohistochem. and imaging of patients with prostate cancer. Novel applications for these antibodies will be discussed.

=> s method
L26 9878122 METHOD

=> s l26 and localizing probe
L27 6 L26 AND LOCALIZING PROBE

=> dup remove l27
PROCESSING COMPLETED FOR L27
L28 4 DUP REMOVE L27 (2 DUPLICATES REMOVED)

=> d l28 1-4 cbib abs

L28 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2002 ACS
1997:369754 Document No. 126:339662 Single molecule detection by in situ hybridization and a digital imaging fluorescence microscopy system. Singer, Robert H.; Femino, Andrea M.; Fogarty, Kevin E. (University of Massachusetts, USA). PCT Int. Appl. WO 9714816 A1 19970424, 49 pp. DESIGNATED STATES: W: AU, CA, JP, KR, MX; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US16811 19961021. PRIORITY: US 1995-546072 19951020.
AB Disclosed are **methods** for accurately detg. the total fluorescence intensity (TFI) of a single fluorochrome, under imaging conditions, using a digital imaging fluorescence microscopy system. Also are **methods** for detecting and **localizing probe** -target mol. binding. The detection **methods** have sufficient resoln. and sensitivity to locate and detect a single target-bound probe bound to a target mol. that can be as short as 20 nucleotides. The **method** is useful in diagnosis for the infection by, e.g., HIV. The **method** was demonstrated by detection of .beta.- and .gamma.-actin mRNA by using .beta.- and .gamma.-actin 3'-UTR probes labeled with fluorescein and CY3, resp., and obsd. with a Nikon DIAPHOT inverted epifluorescence microscope that is capable of digital imaging.

L28 ANSWER 2 OF 4 MEDLINE DUPLICATE 1
96265948 Document Number: 96265948. PubMed ID: 8661803. Endoscopic surgery of the rhinobasis with a computer-assisted localizer. Kruckels G; Korves B; Klimek L; Mosges R. (Department of Ear, Nose and Throat Surgery, Plastic Head and Neck Surgery, Medical Faculty, Technical University of Aachen, Germany.) SURGICAL ENDOSCOPY, (1996 Apr) 10 (4) 453-6. Journal code: VBF; 8806653. ISSN: 0930-2794. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB The endoscope is useful for the diagnosis and surgical therapy of diseases

of the nose, the paranasal sinuses and its neighboring regions, and allows for microinvasive, functional approaches. The reduced invasiveness of therapeutic procedures is sometimes accompanied by insufficient clearness of the surgical field, however. This significant problem is solved by the computer-assisted-surgery (CAS) system, an intraoperative localizer. It allows continuous orientation based on three-dimensional reconstructed preoperative CT scans with superimposed positioning of the endoscope. We have now adapted CAS for endoscopic sinus surgery, which meant that a variety of visualization **methods** were tested. A conventional straightforward endoscope was used in combination with, or as, the **localizing probe**. A dual-display technique was adjusted to video-endoscopic procedures: the information of the localizer is displayed on one monitor while the video-endoscopic picture is viewed on a second screen. In addition, a single-display technique with both images on one monitor was developed. It proved to be the most promising way of combining endoscopy and intraoperative CT-image-guided localization.

L28 ANSWER 3 OF 4 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
94308665 EMBASE Document No.: 1994308665. [Functional endoscopic surgery of the rhinobasis with computer assisted localizer]. ENDOSKOPISCHE NASENNEBENHOHLENCHIRURGIE MIT COMPUTERUNTERSTUTZTER LOKALISATIONSHILFE. Korves B.; Kruckels G.; Klimek L.; Mosges R.. Klinik fur HNO-Heilkunde, Plastische Kopf- und Halschirurgie, Medizinische Fakultat, Pauwelsstrasse 30,D-52057 Aachen, Germany. Oto-Rhino-Laryngologia Nova 4/3 (164-167) 1994.
ISSN: 1014-8221. CODEN: OTNOEQ. Pub. Country: Switzerland. Language: German. Summary Language: German; English.
AB Use of the endoscope can be beneficial for the diagnosis and therapy of diseases of the nose, the paranasal sinuses and its neighboring anatomical regions. However, there is a trade-off between the reduced invasiveness of therapeutic procedures and the reduced clearness of the operative site. We have adapted the Aachen Computer-Assisted Surgery system for use in endoscopic sinus surgery. A variety of visualization **methods** has been tested. With conventional direct view endoscopy, the endoscope can be used in combination with a **localizing probe** or as the **localizing probe** itself. In video-endoscopic procedures a duplex-display technique may be used, where the information of the localizer is displayed on one monitor while the video-endoscopic picture is viewed on a second screen. With the single-display technique, the information derived from online intraoperative localization and from the video-endoscopy are integrated into one monitor screen. The device has been used in 23 cases of endoscopic sinus procedures.

L28 ANSWER 4 OF 4 MEDLINE DUPLICATE 2
80223465 Document Number: 80223465. PubMed ID: 6156040. Fluorescent probes to detect lymphocyte activation. Nairn R C; Rolland J M. CLINICAL AND EXPERIMENTAL IMMUNOLOGY, (1980 Jan) 39 (1) 1-13. Ref: 157. Journal code: DD7; 0057202. ISSN: 0009-9104. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Fluorescent probes can monitor events in lymphocytes stimulated by mitogens and antigens. Early activation is associated with conformational changes in membrane macromolecules, and has been studied by measurement of fluorescence intensity or polarization of the membrane-**localizing probes** ANS, NPN, DPH and TMRITC. Subsequent changes in cytoplasmic macromolecules have been detected by altered fluorescence polarization of intracellular fluorescein. Altered metabolic activity in the activated lymphocyte is also revealed by fluorescent probes: the increased red fluorescence of lysosomes seen by AO staining, is attributable to altered lysosome membrane permeability. AO fluorescence has also detected early changes in the nuclear nucleoprotein complex. The later synthesis of new DNA is readily demonstrated by increased staining with the nuclear probes AO, ethidium bromide, propidium iodide, mithramycin and the Hoechst dyes. Adaptation of fluorescent probe analyses to the now rapidly developing flow microfluorimeters is providing rapid and sensitive assays of lymphocyte stimulation. Such **methods** will permit routine

detection of lymphocyte response to particular antigens or mitogens, as well as identification of antigenic substances by their stimulation of known reactive lymphocytes. Last but not least, fluorescent probes are providing new understanding of the cellular events and regulatory mechanisms associated with lymphocyte activation.

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DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-6.82	-6.82

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